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Abstract

The area of biomedical imaging is fast becoming an active focus for the utilisation of graphene within a variety of imaging modalities. Graphene can be oxidised to produce a material with a high degree of functionality and has led to its expansion as a platform for the immobilisation of fluorescent and radiolabelled molecules. Its large surface area has allowed graphene and its oxides to be modified with a variety of molecules that enhance biocompatibility, selectivity and therapeutic potential. This chapter highlights recent developments in the use of targeted fluorogenic or radiolabelled graphene materials that can be used to image cancers via fluorescence, PET & SPECT modalities. Key emphasis is placed on the nanocomposites that are designed to provide additional therapeutic effects. The capacity of these composites to be internalised by cells and tumours is discussed to appreciate the future perspective of graphene and its congeners as therapeutic multimodal imaging agents.

Keywords: Graphene, Graphene Oxide, Bioimaging, Biosensing, Nanomedicine, Cancer Theranostic, PET, SPECT, Fluorescence, Radiolabelling.

LABELLING OF GRAPHENE, GRAPHENE OXIDES AND OF THEIR CONGENERS: IMAGING AND BIOSENSING APPLICATIONS OF RELEVANCE TO CANCER THERANOSTICS

James A. Tyson, David Gonzalez Calatayud, Vincenzo Mirabello, Boyang Mao

*and Sofia I. Pascu**

Chemistry Department, University of Bath, Claverton Down BA27AY, United Kingdom, email
s.pascu@bath.ac.uk

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Introduction

A. The Problem

Cancer has become a pervasive problem of modern society affecting the health of the global population year on year in an ever increasing manner. Hundreds of thousands of people are diagnosed with cancer every year in the UK alone. It is characterised by over 200 different types, each with its own symptoms, methods of diagnosis and treatment. The latest statistics indicate that in the UK alone there were over 330,000 new cases of cancer in 2011, of which over half of these cases were attributed to prostate, breast, lung and bowel cancer. During the period of 2009-2011, approximately a third of the diagnoses were in individuals above the age of 75.^{1a, 1b}

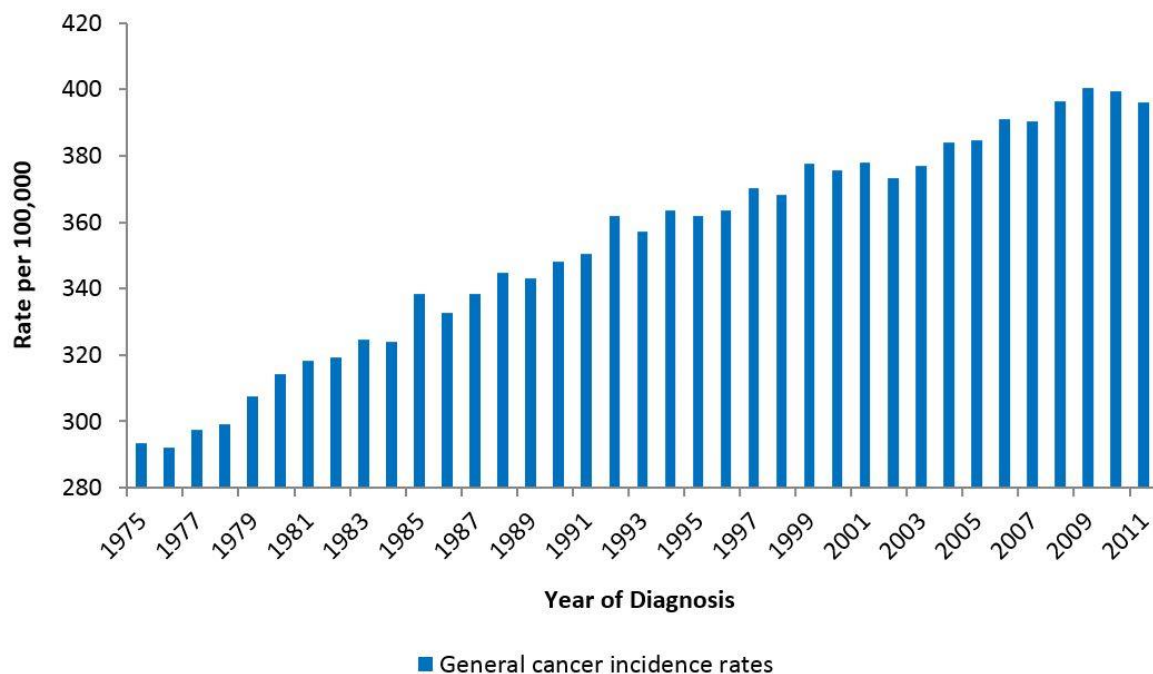


Figure 1: European age-standardised incidence rates for cancers excluding non-melanoma skin cancer, Great Britain, 1975-2011. Data were provided by the Office for National Statistics, ISD Scotland and Welsh Cancer Intelligence and Surveillance Unit on request, May - July 2013.

One of the main reasons behind this increase in diagnosis is due to modern screening and imaging technologies, such as magnetic resonance imaging (MRI) and computed tomography (CT) methods, which have allowed physicians to expose cases of cancer that would have previously remained undiagnosed. The other one is an ever ageing population that has made it necessary to anticipate further rises in cancer incidence that require broad and robust screening technologies in order to inform and assist medical professionals and their patients across the entire spectrum of cancer developments and treatments. Cancer is an extremely complex

degenerative condition, whereby each type of cancer is biologically different from another. Depending on the cell origin and the extent of mutagen exposure, certain cancers will demonstrate varying propensities for proliferation.² For instance, cancers can differ in terms of their tissue origin, with sarcomas arising from connective tissue or muscle, whereas carcinomas come from epithelial cells. Each of these cancers can have a wide array of contributory genetic abnormalities that can influence the overall nature of the cancer malignancy. These factors make it difficult to identify the way the particular cancer cells are behaving and how they are likely to metastasize or damage the body. Without a full understanding of the physiology of the cancer, effective treatments remain elusive to develop. The highly individualised nature of cancer development and wide variation in tumour heterogeneity³ has led to the acknowledgment that an accurate determination of an individual's specific state of cancer progression will allow clinicians to tailor their treatments to suit a particular patient's needs. This will aid in achieving a more personalised approach towards the therapy and diagnosis of cancer.

This is the primary principle of theranostics development whereby screening and diagnosis methodologies simultaneously incorporate therapeutic strategies in order to achieve a more rapid and efficient cancer diagnosis and treatment protocol.⁴

MRI techniques have proven to be a quite effective method in cancer diagnosis due to its ability to provide detailed images of tissue and organs in a comparatively non-invasive manner.⁵ There is a relatively minimal risk to patients associated with use of the MRI contrast agent gadolinium chelate.⁶ However, despite its accuracy and considerable versatility with respect to identifying instances of cancer, it presents a very low sensitivity and there are still issues associated with incidental findings that can complicate the interpretation of resulting images. In addition, to date there are no instances of incorporating simultaneous therapeutic strategies within MRI imaging. On the other hand, it has become necessary to look to alternative imaging techniques and broaden the scope of molecules that have the capacity to not only screen and diagnose, but to also treat cancer to ultimately achieve the theranostics' goals.

Amongst the many several imaging modalities for cancer diagnosis and treatment, fluorescence imaging, Positron Emission Tomography (PET) and single photon emission computed tomography (SPECT) have garnered considerable research interest, due to the

availability of a wide selection of molecules with suitable properties for providing a good signal that can be exploited to image a variety of cancers.⁷

Fluorescence techniques employ a number of well-established molecules directed to target cancer specifically, such as Rhodamine, derivatives of fluorescein and more recently near-infrared emitting cyanine dyes.⁸ The characteristic of these molecules to emit fluorescent and phosphorescent photons after excitation with wavelengths in the visible and near infra-red region of the spectrum has led to their conjugation to a variety of molecules in order to enhance their properties for cancer imaging and treatment. In particular, the use of near infra-red absorbing molecules has transformed the way in which cancer is visualised. Considering that large portions of tissue are transparent to near-infrared wavelengths, such fluorophores have allowed for deeper penetration into living tissue that effectively eliminates the background auto-fluorescence. Furthermore, the capacity for certain fluorophores to undergo intersystem crossing after excitation and achieve triplet excited states has led to their incorporation in simultaneous therapeutic strategies that rely on the generation of cancer targeting singlet oxygen species.⁹

With respect to PET and SPECT imaging modalities, both of these techniques employ radioisotopes as a means of characterising cancers, based on the localisation of intravenously injected radiotracers. Typically, the chosen molecules are labelled with Oxygen-15, Fluorine-18, Carbon-11 and Nitrogen-13 radioisotopes. However, the broad availability of a selection of isotopes within the field of nuclear medicine has led to the development of alternative metallic based radioisotopes with considerably longer half-lives than those of the aforementioned elements. These include various metallic complexes of copper, gallium, indium and technetium amongst others.¹⁰ Although these imaging modalities are very sensitive and quantitative, they possess a poor spatial resolution. The spatial resolution and detection capabilities of PET/SPECT imaging agents are key factors in determining their ultimate value towards cancer diagnosis. Current pre-clinical gamma detecting cameras can provide sub 0.5 mm and 1-2 mm resolution for SPECT and PET scanners respectively. Furthermore, PET/SPECT techniques are now being incorporated into dual MRI or CT systems in order to gather more information about biological systems in the same spatial temporal environment.¹¹

The ability of imaging technologies to achieve both diagnosis and therapy is a considerable challenge that relies heavily on the capacity of the exploited molecules to express an inherent multitude of physical phenomena or to be easily conjugated to molecules that can provide the

additional therapeutic effect. More commonly, the imaging strategy is used to monitor the progress or efficacy of a separately administered therapeutic strategy rather than providing the therapeutic effect as well. However, the field of biological imaging is beginning to expand into the incorporation of graphene materials to achieve just such a goal.

Since its discovery in 2004, graphene and the advent of its associated technologies have seen an incredible rise in research within a multitude of disciplines;¹² from electronics,¹³ materials chemistry,¹⁴ biosensing¹⁵, catalysis¹⁶ and now bioimaging. This is primarily due to its extensive array of impressive properties. Described as a single layer of sp² hybridised carbon atoms arranged in a honeycomb like lattice, graphene is known to possess high intrinsic electron mobility,¹⁷ superior strength,¹⁸ high specific surface area,¹⁹ high thermal electronic conductivity²⁰ as well as interesting optical properties such as high near infra-red absorbance in its oxidised derivatives.²¹ A considerable body of graphene related work for biological applications has utilised the oxidised variants of graphene because of the ease in which large quantities can be produced, superior solvent dispersibility, biocompatibility and ease of functionalisation.²²

Graphene and its oxidised derivatives have proven to be quite versatile materials when it comes to serving as platforms for the immobilisation and delivery of imaging and therapeutic agents *in vitro* and *in vivo*. The abundance of oxygen functional groups on graphene oxide has led to a wide variety of covalent strategies towards the immobilisation of imaging agents and bio-targeting molecules. However, active areas of research are dedicated to methods of immobilisation driven primarily by van der Waals and electrostatic interactions. This can potentially facilitate the formation of an extensive library of molecules aimed at achieving multifunctional Theranostics nanomaterials.²³

The capacity of graphene to provide a surface area and functional group targets makes it an obvious choice for the incorporation of multiple imaging agents, as well as bio-targeting and therapeutic molecules to achieve the Theranostic goals in cancer management. This opens up the potential for graphene as an imaging and therapeutic tool via multiple modalities.

B. Probe Design towards a Solution

The development of graphene oxide based probes, with the aim of achieving imaging via multiple modalities as well as additional therapeutic effects, requires a multifaceted approach to imaging and diagnostic probes' design. A careful selection of molecules exhibiting the appropriate characteristics for imaging via fluorescence, PET and SPECT is required. The

chosen molecule will ultimately determine the method of immobilisation onto graphene oxide, whether it be a covalent or non-covalent strategy. For instance, numerous fluorophore compounds make use of the presence of π conjugation within their structures to attach to graphene through π - π interactions. Additionally, pre-derivatisation of the fluorophore with a biomolecule can eliminate the need for multiple conjugation strategies. In some cases, the strategy involves exploiting the varied charged nature of biomolecules to immobilise them via electrostatics separately. Quite frequently, fluorophores and biomolecules are covalently immobilised via peripheral carboxylic acid groups, which are introduced to graphene after oxidation. Among these molecules that possess fluorescence characteristics, varieties of them contain or can be synthesised to contain metals used in PET/SPECT based imaging modalities.²⁴ Considerable bodies of work are also dedicated to labelling biomolecules with radioisotopes, with the modification of peptide sequences with technetium proving to be an active area of research.²⁵

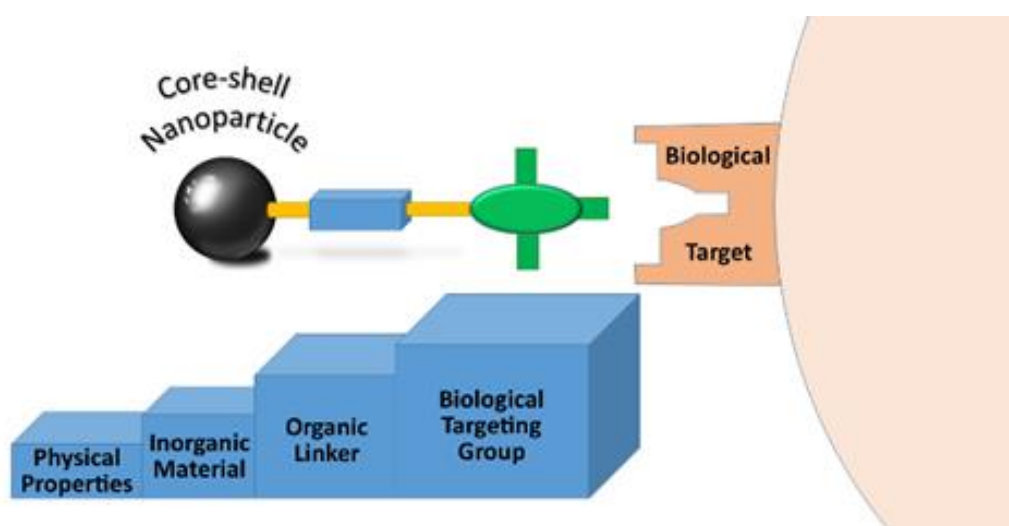


Figure 2: Constituent components of targeted bioimaging probes. (V. Mirabello, D. G. Calatayud, R. L. Arrowsmith, H. Ge and S. I. Pascu, *J. Mater. Chem. B*, 2015, DOI: 10.1039/C5TB00841G - Reproduced by permission of The Royal Society of Chemistry)

With respect to fluorophore attachment to a graphene-based scaffold, a number of studies has exploited the tendency of graphene to act as an efficient quencher of fluorescence via Forster Resonance Energy Transfer (FRET). Some of these fluorophores may also demonstrate the ability to achieve triplet excited states.²⁶ These two phenomena make graphene a potential candidate for the facilitation of photodynamic and photothermal therapeutic effects.²⁷

Multimodal imaging and therapeutic intentions require key design features to be met if a viable imaging agent is to be achieved.

- a) Presence of a suitable signalling agent to facilitate the imaging, such as a radioactive isotope, fluorescent molecule or a combination of both.
- b) A biological molecule to impart cancer specificity onto the intended imaging agent.
- c) An appropriate linking strategy to attach the molecule to the surface of graphene oxide; tailoring or controlling its stability in biological media.
- d) Selection of imaging agent which allows additional phenomena that can be exploited to impart a therapeutic effect
- e) The probe must include a suitable means of retaining stable dispersions of graphene oxide *in vitro/vivo*.

In this Chapter, the imaging agents that constitute the focus of our attention are typically fluorescent molecules or radiolabelled bioconjugates. The majority of the fluorophores discussed possess near infra-red absorbance characteristics and the PET/SPECT capability generally tends to come from the modification of an immobilised molecule with copper, gallium or indium. Particular attention is paid to peptide sequences and their versatility as cancer targeting biomolecules.

Graphene Oxide as a Platform for Fluorescent and Radiolabelled Probes for Cancer Imaging and Therapeutic applications

A. The Chemistry of Graphene and of Graphene Oxides

Graphene is a 1 atom thick carbon allotrope material comprised of sp^2 bonded carbon atoms arranged in a regular honeycomb like lattice structure²⁸. Discovered in 2004 by Andre Geim and Dimitri Novosolev, single and few layer graphene has presented a number of exciting and valuable properties such as high electron mobility in ambient conditions²⁹, tuneable optical properties³⁰, tuneable band gap³¹, high mechanical strength¹⁸ and thermal conductivity²⁰ amongst others.

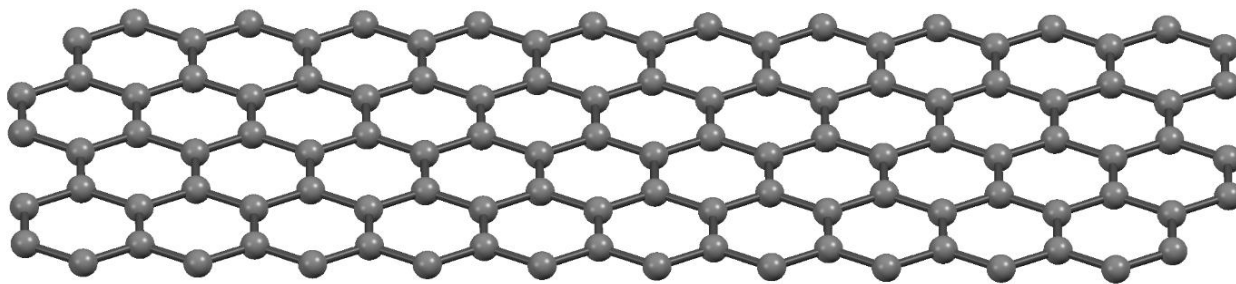


Figure 3: Idealised structure of a pristine graphene sheet containing honeycomb arrangement of hexagonal carbon moieties.

A low cost carbon based material, such as graphene and its oxides, possessing the aforementioned properties, is an ideal candidate material for the development of imaging probes and biosensors for cancer associated with optical methods of transduction. There is a variety of graphene-based materials that are distinguished based on their method of production. Graphene can be produced via chemical vapour deposition (CVD) processes³² and mechanical exfoliation of bulk graphite and graphite oxide³³. Large scale processing of graphene associated nanostructures is achieved by thermal, chemical and electrochemical reduction of graphene oxide.³⁴ Unlike CVD derived or mechanically exfoliated graphene, graphene oxide and its reduced variants possess a significant number of defects as a result of the disruption of the sp^2 bonded planar network of carbon atoms. The oxidation of bulk graphite introduces sp^3 domains as a result of the presence of extensive oxygen functionalities³⁵.

These oxygen functional groups present viable targets for the modification of the material with a variety of molecules which can ultimately serve the purpose of providing a bio-functional material suitable for fluorescent, PET/SPECT imaging and Theranostic applications.

This section will focus primarily on the varied chemistry of graphene oxide and how these aspects influence the immobilisation of optical and targeting agents.

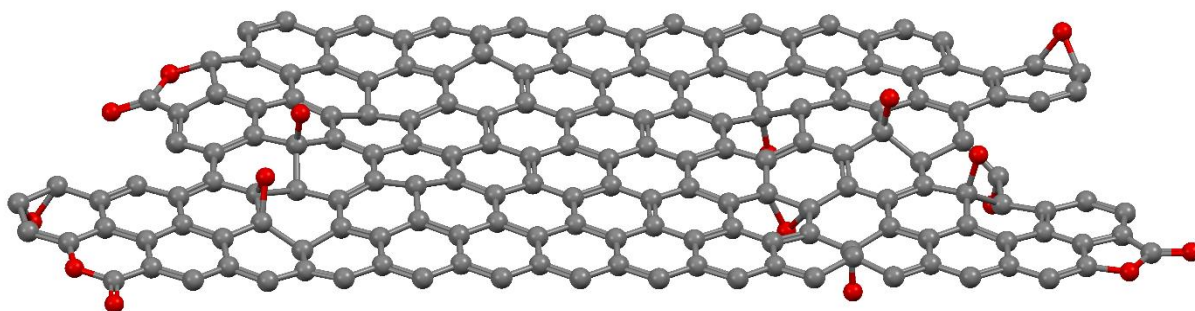
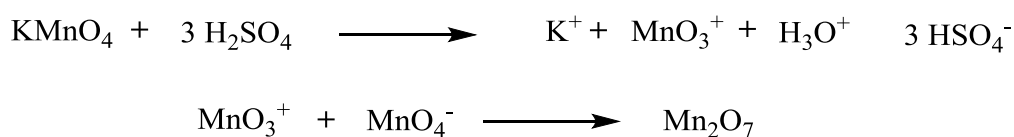


Figure 4: Structure of graphene oxide containing oxygen functionalities across the basal plane and on the periphery.

A considerable percentage of research has been devoted to graphene related materials over the last decade, since the publication of Novosolev and Geim's seminal work reporting a field effect in atomically thin carbon films.³⁶ Therefore, one might assume that the study of graphene materials is a relatively recent area. However, investigations into the chemistry of graphitic materials are thought to precede the 20th century. In 1859, B. C. Brodie performed reactions with flake graphite with a focus towards determining the molecular weight of graphite.³⁷ One particular reaction involved the addition of potassium chlorate to graphite in nitric acid. After four consecutive oxidative procedures, a plateau of oxygen concentration was reached with the resulting material having a Carbon: Hydrogen: Oxygen ratio of 61.04: 1.85: 37.11 and it was readily dispersible in water but not acidic media. It was therefore termed graphitic acid. Latter improvements were made to this method by L. Staudenmaier through the addition of concentrated sulphuric acid and by splitting the addition of potassium chlorate into multiple events throughout the duration of the reaction³⁸. More than half a century after these works, Hummers and Offeman went on to establish the basis for what has now become the most established method for the large scale production of oxidised graphite as a precursor to graphene oxide (GO). In this method potassium permanganate and concentrated sulphuric acid are added in order to generate a dimanganese heptoxide species that acts as an effective oxidant of the flake graphite (scheme 1).³⁹



Scheme 1: Generation of oxidising agent during Hummer's method.

Having established effective methods of oxidising flake graphite, a significant body of work has focused on elucidating a reliable universal structural model for graphene oxide. This has proven to be a particularly challenging task, in large part due to the variation in atomic content of flake graphite derived from its amorphous berthollide mineral source. A further barrier to determining a general structure is the fact that the GO materials studied are usually derived from processes having modifications to the Hummers method, which vary from procedure to procedure. The following section describes some of the more established models developed for graphene oxide and the associated techniques used to establish the nature of its oxygen functional groups.

The first concerted approach to developing a structural model for GO was performed by Hofmann and Holst. The structure they proposed contained repeating lattice units with epoxy groups littered across the basal plane of graphite conforming to a sp^2 hybridized system. Reuss *et al* developed this model further by introducing hydroxyl groups into the structure proposed by Hofmann and Holst. This was also accompanied with a change in structure of the basal plane from a sp^2 hybridised system to that conforming to a sp^3 hybridisation.⁴⁰

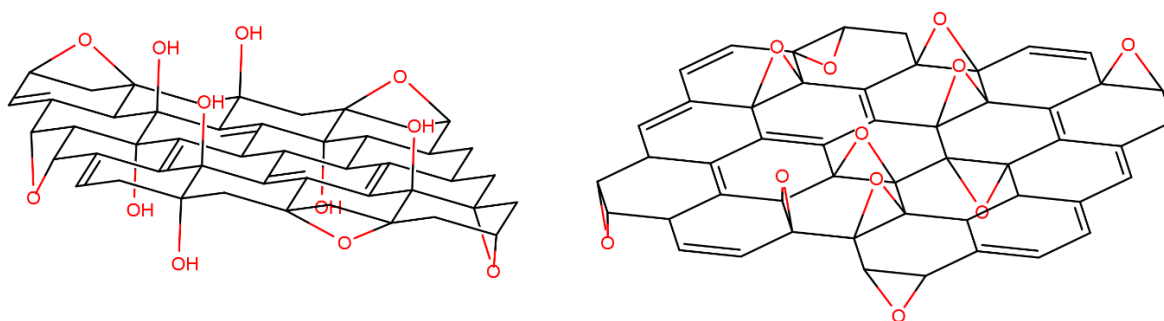


Figure 5: Hofmann and Reuss structures proposed for GO, the former containing epoxides and the latter containing both epoxides and hydroxyl groups.

This model retained the repeating unit nature of the Hofmann and Holst model, but the distinguishing feature was the suggestion of epoxide groups in the 1 and 3 positions of the cyclohexane moieties as well as hydroxyl groups in the 4th position.

Latterly, Scholz & Boehm and Nakajima & Matsuo proposed slightly differing structures, with one possessing quinoidal species without epoxides or ethers⁴¹ and the latter containing a stage two graphite intercalation structure⁴². Beyond these models, Lerf and Klinowski have proposed a different structure. They performed extensive solid state Nuclear Magnetic Resonance (NMR) investigations on the products of various reactions with graphene oxide in

order to extract information regarding hydration characteristics and the material's reactive functional groups. The key feature noted from these investigations was the suggestion of the presence of tertiary alcohols, epoxy groups and various alkenes evidenced by resonances situated around 60, 70 and 130 ppm respectively. Their work also explained the tendency for GO to stack due to the presence of hydrogen bonding facilitated by the alcohol and epoxide groups between the platelets of GO.⁴³ This model largely conformed to previous models differing only in the suggestion that the ethers existed in the 1 and 2 positions as opposed to the 1 and 3 positions as suggested by earlier models. Lerf and Klinowski also re-evaluated the conclusions derived from the earlier spectroscopic studies to suggest that in addition to epoxides and tertiary alcohols, carboxylic acid groups also existed in relatively smaller amounts on the edges of graphitic planes.⁴⁴

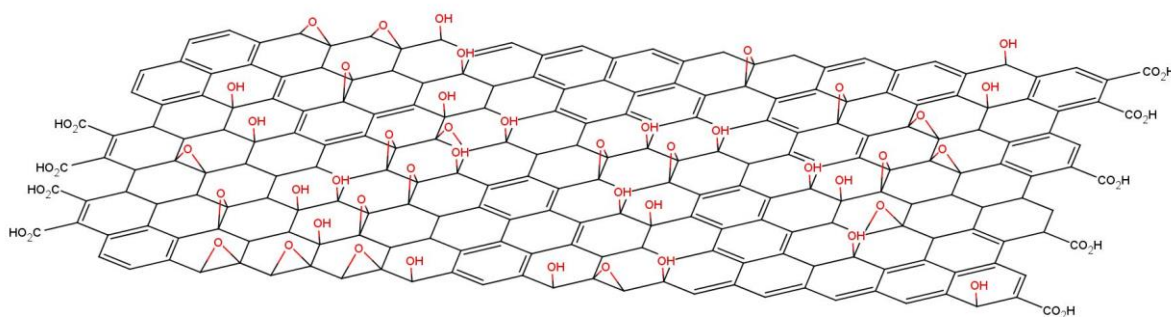


Figure 6: Lerf and Klinowski model containing epoxides, tertiary alcohols and carboxylic acid groups on the edges of GO.³⁴

Facile and rapid routes to functionalising graphene oxide for biomedical applications

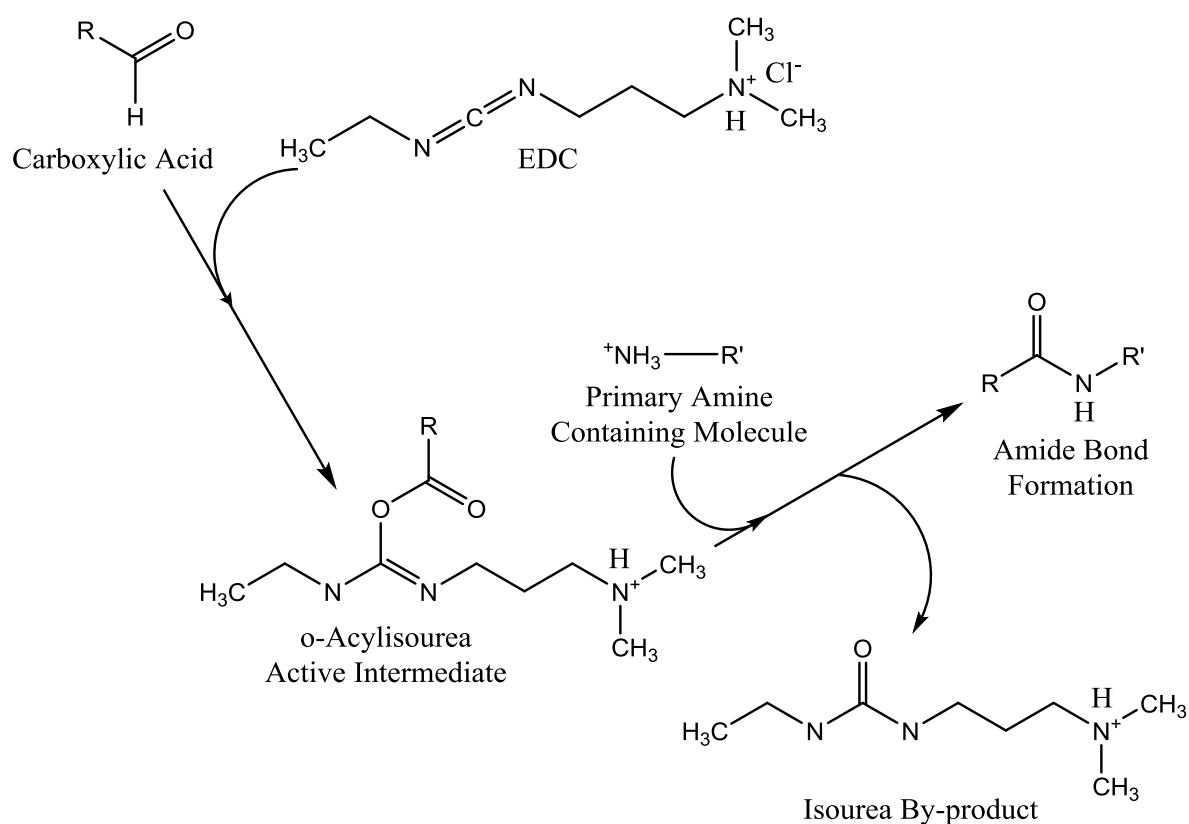
Having established the presence of a variety of oxygen functional groups on GO, the material naturally becomes a promising candidate for various chemical functionalisations that would render it a far more valuable material for biomedical imaging and sensing applications.

Carboxylic acid activation

When considering the use of carboxylic acid groups as targets for functionalisation it is necessary to use an appropriate activating agent. Thionyl Chloride,⁴⁵ EDC (EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide),⁴⁶ DCC (*N,N'*-dicyclohexylcarbodiimide) and HATU (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) are just some examples of compounds used for this purpose.⁴⁷ Then, molecules containing amines and hydroxyls can be added to form amide or ester linkages. The

use of carbodiimides has proven to be a popular choice in regards to the conjugation of biomolecules to a variety of surfaces. This is in part due to this particular reagent facilitating the formation of what is known as a zero length cross-linker. In this methodology, two molecules are attached to each other via the formation of a bond that contains no extra interfering atoms.⁴⁸ This is desirable in regards to the final application, as additional spacing atoms between two molecules may hinder the ultimate function of the hybrid molecule, which is of vital concern when establishing functional biomaterials. Carbodiimides are therefore a widespread choice because they are able to facilitate the creation of amide linkages between species containing carboxylic acids and amines. They are considerably versatile reagents in that they can be used to effectively couple peptide sequences, proteins and other complex biomolecules to each other as well as a variety of surfaces.

EDC is one of the most widely used carbodiimides for coupling reactions involving biomolecules. EDC will react with a carboxylic acid containing molecule to create a stable ester intermediate. The addition of a nucleophilic amine containing molecule facilitates the formation of an amide bond with the loss of an isourea by-product.



Scheme 2: Schematic representation of reaction scheme to produce amide bonds via EDC mediated coupling adapted from reference 48.⁴⁸

The primary concern with the use of this reagent is the potential for self-polymerisation in which the bio-molecule may react with another molecule of itself due to the presence of carboxylates and amines.

Studies have been performed whereby carbodiimide activation approaches have been used to modify GO sheets with polyethylene glycol (PEG) as a means of developing a biocompatible drug delivery vector/vehicle for anti-cancer drugs.⁴⁹ In this study *in vitro* tests were carried out in which it could be to demonstrate that the covalently functionalised GO served as a viable vector for the immobilisation of anticancer drugs via π - π stacking. The GO hybrid material was ultimately shown to possess a high performance towards the killing of human colon cancer cells.

Carboxylic activation methods have also been used for the covalent attachment of porphyrins to GO as a means of developing light harvesting materials for photovoltaic applications.⁵⁰ The overall broad applicability of carboxylic activation via carbodiimide mediated or alternative activation approaches demonstrates the suitability of these reagents for the development of zero length cross-linkers for fluorescent and bio-electronic applications.

Epoxide ring opening reactions

As discussed earlier, a significant number of structural models based on infra-red spectroscopic studies and solid state NMR investigations have suggested the existence of epoxy groups distributed along the basal plane of the graphitic domains. These are in a greater abundance with respect to the carboxylic acids and therefore present another attractive area for molecular functionalization.³⁴

In 2011, one study utilised an epoxide ring opening strategy with an amine containing ionic liquid in order to provide a suitably positive electrostatic charge for the immobilisation of a glucose oxidase enzyme.⁵¹ A relatively simple methodology in which the ionic liquid [1-(3-aminopropyl)-3-methylimidazolium bromide] (NH₂-IL) was added to a GO dispersion along with potassium hydroxide and with 24 hours of stirring overnight, led to the subsequent immobilisation of glucose oxidase into the IL-graphene surface. Ring opening strategies have also been used to impart photoluminescent properties onto graphene oxide.⁵² In this work alkylamines of varying chain length were used to remove the opportunity of recombination of electron-hole pairs as a result of the presence of both epoxy and carboxylic acid groups, which is largely seen as the reason for the lack of strong fluorescence emission characteristics in GO.⁵³

Acylation reactions to form surface amides followed by nucleophilic ring opening aminations lead to the removal of both the carboxylic acid and epoxy groups, which in turn greatly enhanced the fluorescent quantum yields of GO.

Non-covalent tagging strategies

The extensive sp^2 hybridisation of the planar graphitic domains opens up the opportunity for functionalisation via π - π stacking or van der Waals forces in the areas that lack the oxygen functionality or hydrogen bonds.

Hunter and Sanders have described a model for understanding the interaction between separate aromatic systems. In general, the electron density derived from the π orbitals of aromatic rings creates a quadrupole moment possessing an overall partial negative and partial positive charge on top of aromatic faces and on the periphery respectively. When these moments come within a suitable distance, a face centred parallel stacking interaction can occur.⁵⁴ The interaction between aromatic molecules that alternate in their extent of electron density is sometimes referred to as aromatic donor-acceptor interactions. Although there is still considerable debate in regards to the driving forces behind the interactions between aromatic molecules in close proximity to one another,⁵⁵ it would still suffice to conclude that such interactions present an attractive strategy towards the construction of supramolecular assemblies based on aromatic graphitic and fluorescent bio-molecules.

We have recently focused our research work on non-covalent strategies to functionalise the surface of GO with aromatic chromophores in particular with aryl thioacetate Zn(II) porphyrin. Porphyrins are aromatic, planar and electron-rich molecules well known for their high extinction coefficients and near infrared (NIR) emissions. A supramolecular self-assembly, based on donor-acceptor interactions, results in a new class of GO@Zn(II)-porphyrin nanohybrid forming a stable dispersion in the most common organic solvents. The morphology of such a supramolecular complex has been extensively investigated by atomic force microscopy (AFM) and transmission electron microscopy (TEM). These analyses, together with UV-Vis titrations, have demonstrated that a ground state Zn(II) porphyrin complex can interact with the π orbitals of aromatic rings of GO. On the other hand, fluorescent spectroscopy and time correlated single photon counting carried out on GO@Zn(II)-porphyrin complex and Zn(II) porphyrin have suggested that, upon excitation, an excited state Zn(II) porphyrin can also interact with GO forming a stable complex via FRET. The use of a NIR dye capable of

generating FRET in the presence of GO *nanosheets* can certainly be of great interest in the development of new forms of imaging/therapy probes.

Various studies have now led to a beginning of an understanding of the complex mechanisms by which graphene can be internalised by a variety of mammalian cells. Uptake of graphene *nanosheets* has been studied by Portoles *et al.* in which osteoblasts, hepatocytes and macrophages were incubated with PEGylated^a GO *nanosheets* modified with an fluorescein isothiocyanate (FITC) dye to trace their localisation *in vitro*.⁵⁶ Cells were incubated with the GO conjugates, along with a number of endocytosis inhibitors in order to understand the pathways that influence the migration of GO into cells. They were able to show that micropinocytosis is the most prevalent means of uptake. Macropinocytosis is a process in which a fluid containing vesicle, known as a micropinosome, breaks away from the cell surface and incorporates the material in question.⁵⁷ The micropinosome along with its cargo is subsequently drawn into the interior of the cell. In addition to micropinocytosis, phagocytosis, microtubule and clathrin dependent mechanisms can also play a role in graphene endocytosis. Ultimately this study showed that there is a general method of cellular internalisation; however, there can be additional mechanistic pathways depending on the cell type.

Nano-graphene oxide, i.e. a graphene oxide whose 3 dimensions are under 100 nm, has been reported as a viable facilitator of drug delivery and cellular imaging applications. In 2008, a study was carried out in which PEGylated nano-graphene oxide was used to image cells and deliver anti-cancer drugs. The GO materials that possessed fluorescent properties in the visible and near-infra red region were used to selectively image Raji-B cells via the detection of near infra-red photoluminescence.⁵⁸ Furthermore, the physical adsorption of the anti-cancer drug doxorubicin via π - π interactions onto the *nano* graphene oxide with a cancer targeting antibody Rituxan was able to facilitate the selective killing of specific carcinoma *in vitro*.

In summary, GO presents a number of options for functionalisation via various covalent and supramolecular interactions. The broad applicability of the resulting nano-materials from fluorescent materials, drug delivery vectors, electrochemical through to light harvesting applications enhances its viability as a candidate for the development of multifunctional imaging and therapeutic tools that exploit optical transduction methodologies.

^a PEGylation is the process of both covalent and non-covalent attachment or amalgamation of polyethylene glycol (PEG) polymer chains to molecules and macrostructures, such as a drug, therapeutic protein or vesicle, which is then described as PEGylated

B. Fluorescence imaging and therapeutic applications of functional Graphene Oxide-based probes

A fluorescent probe is a compound that possesses an intrinsic ability to emit fluorescent photons after excitation with an appropriate wavelength. Upon conjugation to a biomolecule via a reactive site on the fluorophore, the resulting modified biomolecule can be suitable for applications such as imaging and fluorescent based biosensors. Labelling biomolecules with fluorescent compounds is seen as an attractive option for the aforementioned applications because of their highly specific emission characteristics after excitation with light.⁵⁹

The processes that occur in fluorescence during the absorption and emission of light can be illustrated by the Jablonski diagram (Figure 7).

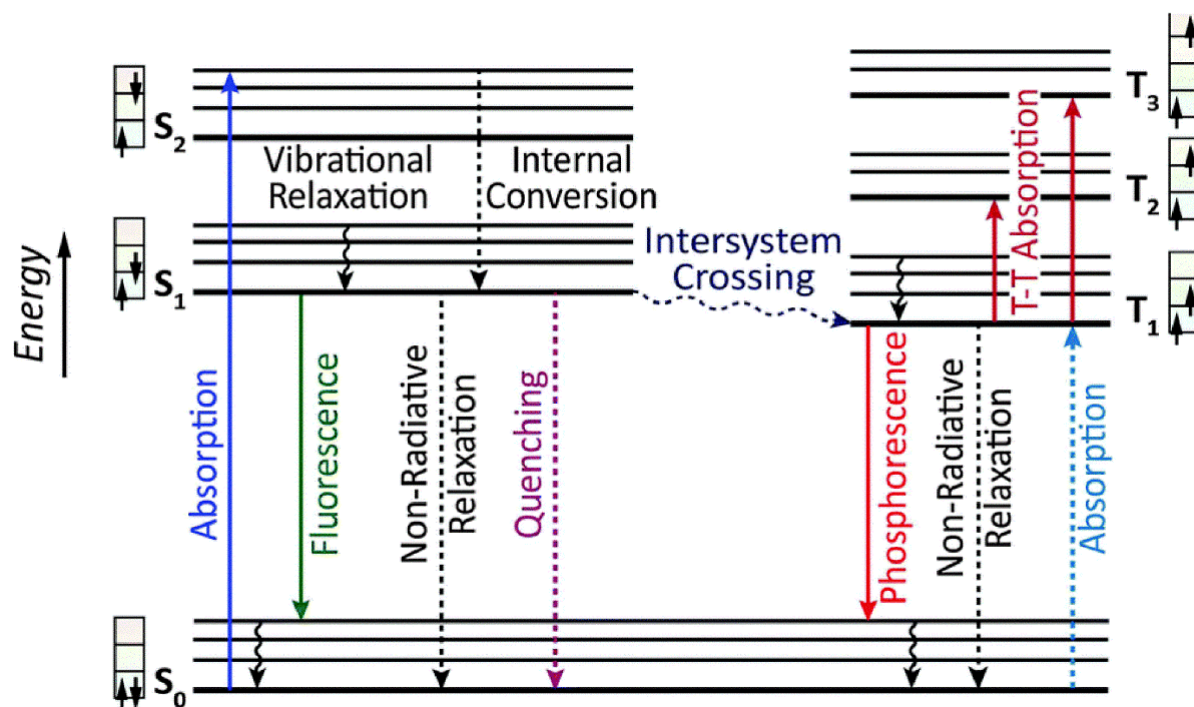


Figure 7: Jablonski diagram illustrating the various radiative and non-radiative decay processes occurring after excitation. (H. Xu, R. Chen, Q. Sun, W. Lai, Q. Su, W. Huang and X. Liu, Chemical Society Reviews, 2014, 43, 3259-3302 - Published by The Royal Society of Chemistry)⁶⁰

The initial transition denoted in the Jablonski diagram is the process of photon absorbance. During this event a photon of a specific energy is excited from a lower ground energy level to a higher energy state. The photonic energy is transferred to an electron, which then excites to

a heightened state depending on the energy transferred. The absorbance events occur quite rapidly in the region of 10^{-5} seconds.⁶¹ Once an electron has reached an excited state, the energy absorbed can be dissipated through the loss of a photon. This event is known as fluorescence and occurs at relatively slower timescales such as 10^{-9} to 10^{-7} seconds. Fluorescence phenomena tend to occur between the 1st excited electron level and the ground state. At higher energies, dissipation of the absorbed energy is more likely to occur via internal conversion or vibrational relaxation. During vibrational relaxation, the energy absorbed during excitation can be given to other vibrational modes as kinetic energy. Internal conversion also occurs when vibrational energy levels overlap with electronic energy levels allowing for transitions between vibrational states of different electronic levels.⁶¹ Finally, intersystem crossing can occur in which electrons in a singlet state transition to an excited triplet state. The transition down from an excited triplet state to a singlet ground state is responsible for phosphorescence phenomena. It is such excited state energy transitions that allow for photodynamic therapeutic strategies or fluorescence resonance energy transfer based *in vitro/vivo* recognition and imaging methodologies that will be the main focus of the first section of this review.

C. Peptide Sequences for the Specific Targeting of Cancer and means to their incorporation into GO

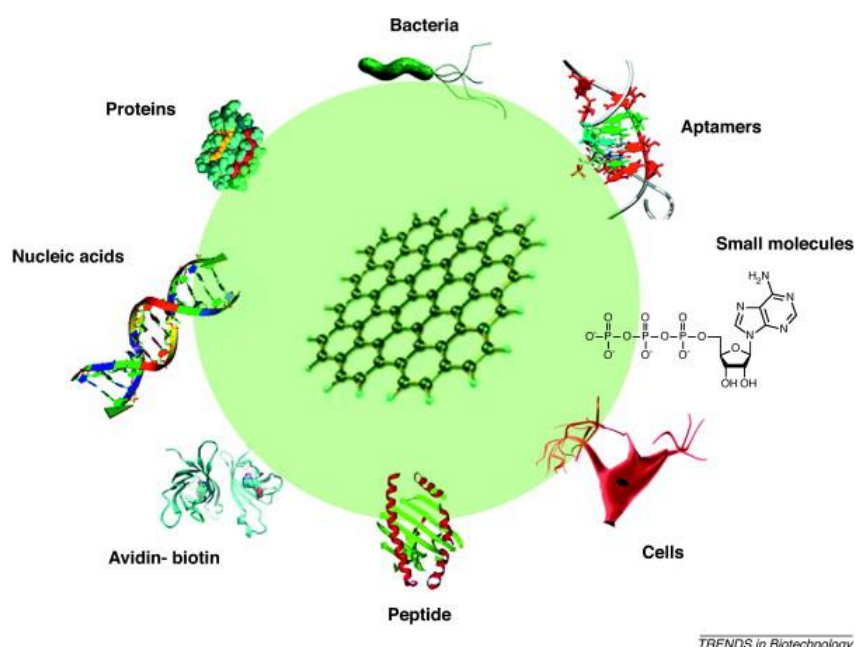


Figure 8: Scope of molecules available for graphene modification. (Reprinted from Y. Wang, Z. Li, J. Wang, J. Li and Y. Lin, Trends in Biotechnology, 2011, 29, 205-212, Copyright (2011), with permission from Elsevier)^{1c}

During the process of developing functional nanomaterials for biological applications, it has been shown that it is essential to include in the probe composition a biomolecule to engender specificity. Over the years, various antibodies, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules have been used to derivatise surfaces and other small molecules as a means of developing bio-targeting surfaces and molecules for applications ranging from electrochemical biosensors, drug delivery vehicles, radiolabelling and fluorescence imaging.⁶²

A significant body of work has focused on the use of peptide sequences as molecular targets for cancer Theranostics.^{63,64} Peptide sequences are seen as an alternative to antibody based targeting strategies due to their considerably smaller size in relation to their antibody counterparts used for similar applications. The large molecular weights of antibodies tend to result in difficulties when targeting large tumour masses and there are also issues associated with their non-specific uptake by the liver, spleen and bone marrow which further limit their effectiveness.⁶⁵ Peptides are sequences of amino acids that can vary widely in size and function based on the number and type of amino acids attached to each other. Part of the reason for the utility of peptides in cancer Theranostics is associated with their ability to target specific receptors that are expressed on the cellular membranes of a variety of cancers.

Recent work has demonstrated that relatively small peptides tend to assemble towards the edges or planar surfaces of graphene oxide via π - π interactions.^{66,67,68} Katoch *et al.* carried out studies in which they were able to show that the peptide sequence of GAMHLP-WHMGTL^b transformed from an α -helix to a reticular structure when adsorbed on graphene surfaces.⁶⁹ Atomic level simulations have also demonstrated that peptides which possess α -helices tend to unfold and form amorphous dimers upon conjugation to graphenes. Studies have suggested that the phenomenon of α -helix unfolding begins at the C-terminus of the peptide and the degree of unfolding is largely influenced by the strength of the interactions between certain amino acids.

Density functional theory (DFT) studies have also been used to develop a deeper understanding regarding the interaction of graphenes with amino acids and peptides. Kawazoe *et al.* have modelled the interaction of phenylalanine, histidine, tyrosine and tryptophan with graphene and carbon nanotubes. Their results suggest that the aromatic components of these

^b A peptide sequence ZP-1. Mammalian zona pellucida (ZP) is an extracellular matrix surrounding and protecting mammalian and fish oocytes, which is responsible for sperm binding. ZP consists of three to four glycoproteins, called ZP1, ZP2, ZP3, ZP4

amino acids re-arrange themselves to a parallel conformation along the basal plane of the graphitic domains via π - π interactions. In addition to these theoretical studies, experimental investigations have probed the interaction of lysine, arginine, tryptophan and tyrosine with GO. They have also concluded that binding between amino acids and GO can be considerably influenced by π - π interactions. However, they also suggest that the positively charged side chains on lysine, histidine and arginine can bind to GO via additional electrostatic mechanisms. In general a large majority of studies associated with experimental and atomistic investigations have implicated the amino acid residues such as tryptophan as being the strong driving force behind the immobilisation of peptides to graphene and carbon nanotube surfaces.^{70,71,72}

GO materials based on immobilised peptides are showing significant potential towards developing multifunctional and highly sensitive detection platforms. Recently dye-labelled peptides immobilised on GO surfaces have shown considerable promise towards developing multifunctional and highly sensitive detection platforms. The first reported study of a GO based sensing mechanism that utilised a non ssDNA molecule as a bio-recognition molecule was reported in 2011. In this work, a peptide labelled with fluorescein isothiocyanate (FITC) was used to target the serine protease thrombin.⁷³ The use of this fluorophore facilitated fluorescence resonance energy transfer between the GO and the fluorescent peptide, allowing for the monitoring of protease activity. This work was able to demonstrate that the increase in fluorescence intensity had a direct correlation to the concentration of Thrombin. The fluorescence based detection mechanism allowed thrombin to be quantified in concentrations as low as 2 nM. Additionally, a fluorescence-based detection mechanism for Caspase-3 (a known mediator for the triggering and spread of apoptosis events) was recently developed. In this research a peptide sequence of Asp-Glu-Val-Asp with a fluorescein amidite (FAM) labelled lysine was bound to GO surfaces using EDC/NHS (EDC = 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS = N-Hydroxysuccinimide) activation approaches to produce a novel GO fluorescent peptide conjugate (Figure 9).⁷⁴

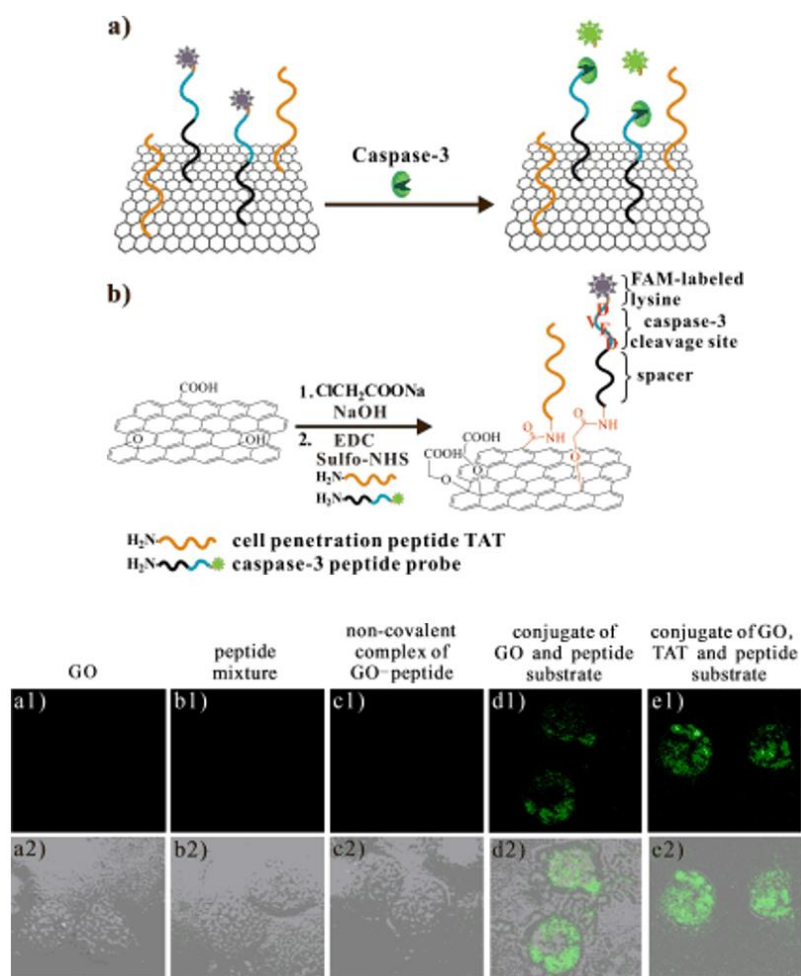


Figure 9: Caspase-3 detection using GO-peptide conjugate and confocal fluorescence microscopy images of HeLa cells treated with 4 μM STS for 4 h after incubation. (Reprinted from H. Wang, Q. Zhang, X. Chu, T. Chen, J. Ge and R. Yu, *Angewandte Chemie International Edition*, 2011, 50, 7065-7069 Copyright (2011), with permission from Wiley)⁷⁴

The bio-fluorescent GO hybrid material was successfully taken up by cells, followed by cleavage of the dye labelled peptide by internal proteases. Cleavage of the bond released the dye labelled peptide from the quenching effects of GO allowing its fluorescence characteristics to be used as means of verifying and quantifying the presence of Caspase-3. The bio-conjugate material demonstrated a high degree of stability in water and cell growth media and a wider promise towards the applicability of peptide-GO conjugates as cancer sensing mechanisms. The majority of the examples discussed herein will focus primarily on peptide based methods of bio-targeting and how this ultimately influences the distribution of the probe *in vitro/vivo* and the effectiveness of the imaging modality.

D. Targeted Fluorescent Molecules anchored onto Graphene Oxide for Cancer Imaging and Therapy Applications

Graphene has demonstrated a capacity to serve as an imaging, diagnostic and therapeutic platform in a variety of functional states. In some instances, it can be used in conjunction with other nanoparticles to form effective diagnostic tools. In 2014, Ji *et al.* developed a prognostic indicator for early-stage cancer based on a combined graphene-mesoporous silica nano-sheet.⁷⁵ The value of such a system with respect to the *in situ* detection of prognostically significant bio-markers was fully realised after assembling the graphene-silica (GS) platform with a polyethylene glycol derivatised hexapeptide (PEG-RWIMYF) that served as recognition mechanism cyclin A₂ (Figure 10). This particular cyclin has been implicated in the deregulation of cyclin dependant kinase (CDK) activity and hence chromosomal instability and ultimately tumour proliferation.⁴⁴ Initially, the GS surface was functionalised with amines which were then converted to carboxyls (COO⁻) to allow for the electrostatic capping adsorption of the positively charged peptide. Prior to this step, the mesoporous silica had been loaded with Rhodamine B.

Upon specific binding to cyclin A₂, the peptide capping agent was removed from the GS nano-surface allowing for the release of Rhodamine B, effectively acting as a turn on switch for the quenched fluorescence.

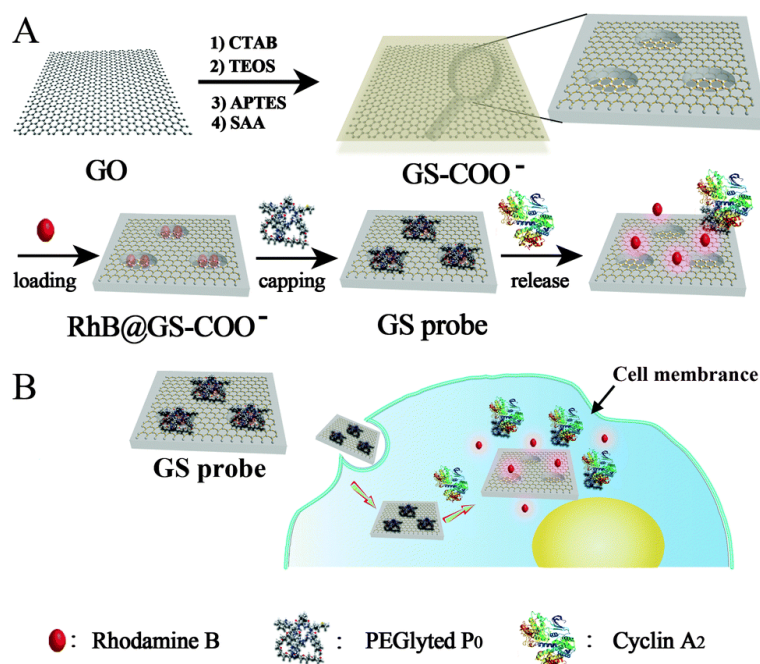


Figure 10: Schematic illustration of the GS-based peptide probe for cyclin A₂ detection. (Reproduced from Ref 75 with permission of The Royal Society of Chemistry)⁷⁵

The tendency for graphene to act as an effective quencher, as well as its capacity to be functionalised to facilitate physisorption, allowed for the efficient intracellular delivery of the fluorescence based recognition imaging agent. This particular functional nano-system was able to demonstrate the *in vitro* concentration dependant detection of cyclin A₂ in a variety of cancerous and healthy cell lines based on the observed fluorescence intensity. Furthermore, the probe could be used to monitor the efficacy of the anti-cancer drug doxorubicin (DOX). DOX is known to up-regulate the expression level of cyclin A₂. Consequently, in cell lines that were treated with DOX a corresponding increase *in situ* fluorescence was observed.

This multicomponent approach to graphene probe design is one example of how incorporating peptides and fluorophores opens up the opportunity to exploit cellular recognition mechanisms to not only image and detect diagnostically relevant biomarkers, but to also track the progress of therapeutic strategies.

One of the more widely researched biomarkers for the screening of prostate cancer is Prostate Specific Antigen (PSA). PSA is a serine protease that is produced by both prostate carcinoma, as well as their healthy or benign counterparts. Currently, there is still a great deal of limitation associated with PSA diagnostic measures in that they regularly fail to provide a suitable degree of specificity with respect to identifying and distinguishing between prostate cancers based on their malignancy.⁷⁶ The proteolytic active form of PSA has been shown to be a more effective discriminator of prostate cancers based on their metastatic potential.⁷⁷ This has led to the development of fluorescence based peptide targeting methods that can facilitate the “on-off” switching signal for this particular active form of PSA. Feng *et al.* developed an imaging based sensing probe through the modification of GO via peptide derivatised fluorescein isothiocyanate.⁷⁸ The peptide containing the sequence HSSKLQ^c has been shown to be selectively cleaved by the proteolytic form of PSA. The construction of this sensing probe was achieved via the π - π and electrostatic interactions between peptide labelled FITC and the GO surface. As the active PSA is bound to the nano-construct, the protease would cleave the peptide allowing for the release of the dye due to diminished electrostatic interactions and hence recover the FITC fluorescence that was quenched due to the close proximity of the GO.

^c A peptide sequence that is selectively cleaved by the proteolytic form of the prostate specific antigen

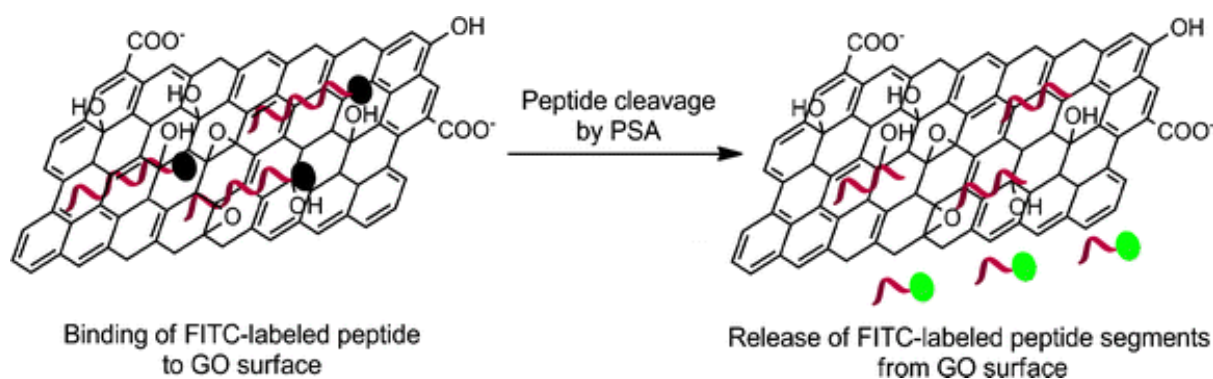


Figure 11: Schematic illustration of FITC labelled peptide conjugate loading on GO surface. (Reproduced from Ref 78 with permission of The Royal Society of Chemistry)⁷⁸

This study showed that a rapid fluorescence response could be observed when the GO dye labelled peptide was exposed to varying concentrations of PSA, with a detection limit of 0.3 nM. Quite crucially, they were able to demonstrate the effective detection of active PSA in urine samples despite the presence of a variety of interfering molecules such as CaCl₂, MgCl₂, *L*-histidine and glycine amongst others. This study ultimately showed that exploiting the charge transfer capacity of GO to induce quenching, can be used in conjunction with targeted dye based systems for the effective “on-off” detection/sensing of diagnostically relevant biomarkers.

The utility of combined fluorophore, peptide and GO constructs has extended to the monitoring of peptide receptor interactions. Somatostatins are hormone receptors that play a role in the regulation of the endocrine system. They have been implicated in cell proliferation and are therefore of particular interest when aiming to characterise a variety of cancers.⁷⁹ Octreotide is an 8 amino acid cyclic peptide sequence consisting of FCFWKTCT^d. It is known to serve as an effective ligand for somatostatins and as such has led to its employment in graphene based recognition systems for imaging and sensing. Bianying *et al.* developed an imaging based sensory platform to characterise the aforementioned peptide-receptor interactions based on the combination of a FITC dye labelled octreotide (FOC) (Figure 12).⁸⁰ The FOC conjugate was adsorbed to the surface of GO and enhanced via the positively charged nature of the lysine residue, as well as the π - π interactions associated with the tryptophan and phenylalanine residues. The presence of these particular residues demonstrated that the FOC molecule could readily bind to the surface of GO with efficient quenching and adsorption

^d Octreotide an 8 amino acid cyclic peptide sequence. It is known to serve as an effective ligand for somatostatins and as such has led to its employment in graphene based recognition systems for imaging and sensing

kinetics. After exposure of FOC to its antibody counterpart (anti-octreotide – AOC), the corresponding competitive interaction forced the release of FOC from the surface of GO allowing for the re-emergence of its fluorescence.

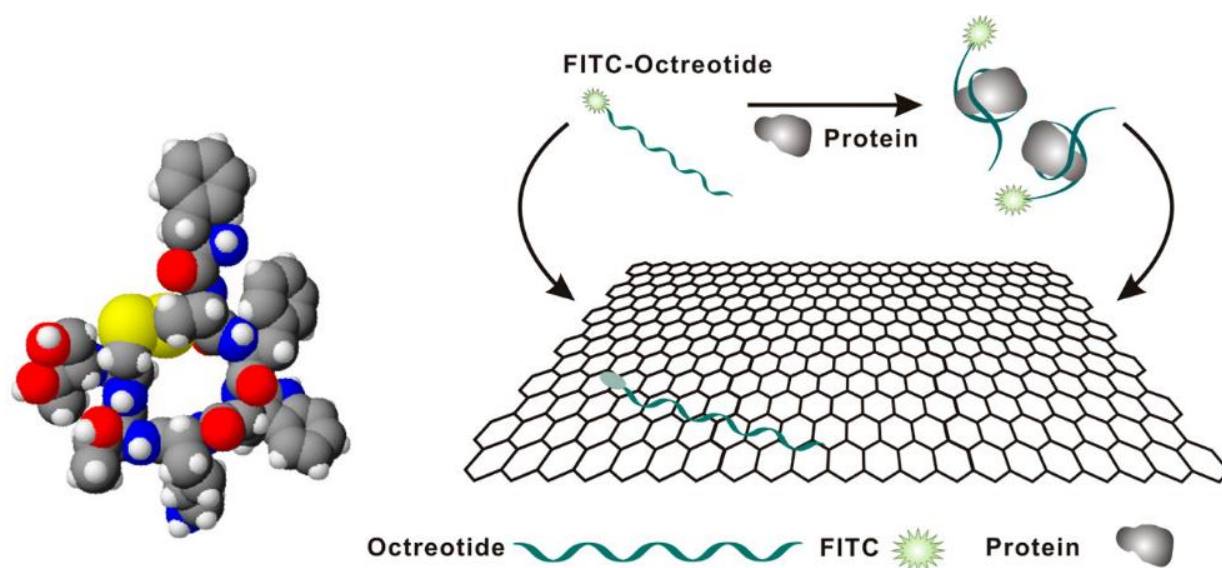


Figure 12: 3-D Structure of Octreotide and schematic illustration of the concept of using FITC–Octreotide and GO to detect peptide–receptor interactions. (Reprinted with permission from F. Bianying, G. Linjie, W. Lihua, L. Fan, L. Jianxin, G. Jimin, F. Chunhai and H. Qing, *Analytical Chemistry*, 2013, 85, 7732-7737. Copyright (2013) American Chemical Society).⁸⁰

A greater signal to noise ratio was also noted for the total observed fluorescence *in vitro* when GO was included in the probe construct to suppress cellular auto-fluorescence. A linear relationship of AOC detection was observed based on the total fluorescence intensity to concentrations as low as 2 ng/ml. This specific peptide has been used to image the cancer cells and track the movement of the biomolecular interactions. AR42J rat pancreatic cells with a high expression of somatostatin receptors showed strong *in vitro* fluorescence upon release of FOC from the GO surface after receptor binding. This was in stark contrast to the significantly diminished fluorescence in CHO cell lines that possess a lower expression of somatostatin receptors. Ultimately, the study demonstrated that the combination of specific peptide interactions in conjunction with dye and GO molecules can serve to image and sense biomolecular interactions that are relevant to cancer development.

One of the key challenges of developing Theranostic imaging agents for cancer based on GO materials is associated with enhancing the ease at which it can be dispersed in biological media. Many studies have utilised PEGylated GO to achieve just this purpose. However, the

utility of dendrimer type molecules extends to not only enhancing the stability of GO dispersions, but to also providing additional functional group anchor points. Dendrimers can serve to provide a branched architecture with a variety of functional groups for the immobilisation of a number of imaging and targeting agents.⁸¹ Wate *et al.* developed a GO system in which the dendrimer PAMAM G4 was used as an interface to facilitate the incorporation of a cyanine dye and magnetic Fe_3O_4 nanoparticles.⁸² The inclusion of magnetic nanoparticles enhanced the capacity to direct the cellular localisation via the application of an external magnetic field. This work focused primarily on covalent methods to develop the nano-construct. Glutathione (GSH) was used as a spacer/linker molecule to attach Fe_3O_4 and a cyanine dye to the GO surface via EDC carbodiimide activation protocols.

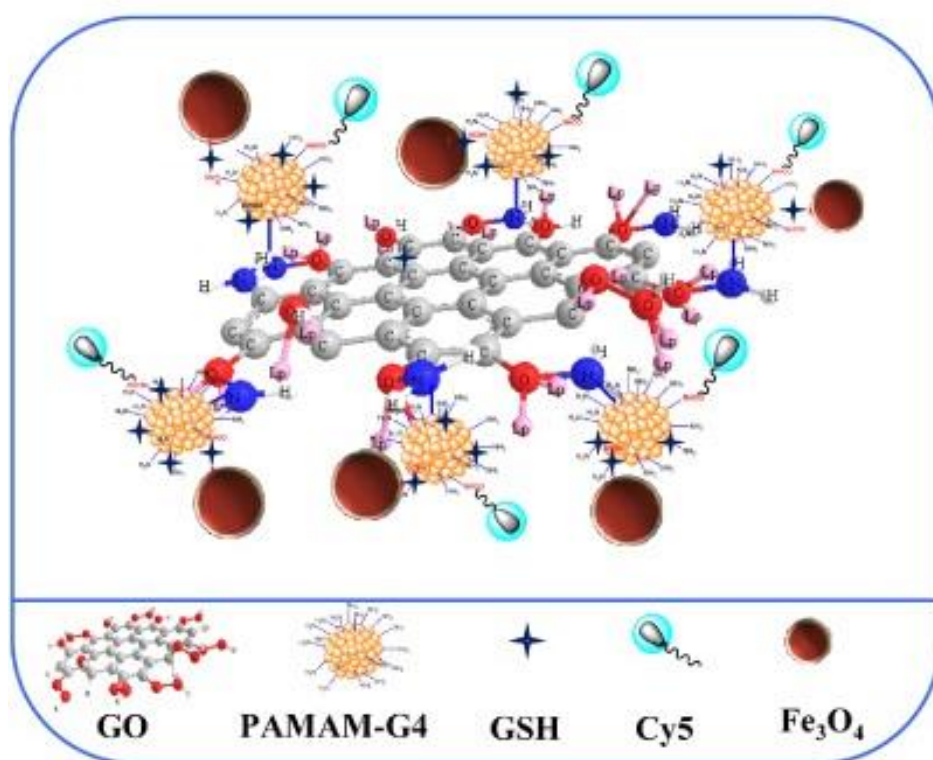


Figure 13: Schematic of multicomponent magneto-dendritic graphene oxide nanosystem consisting of PAMAM G4 dendrimer (G4), Cyanine 5 (Cy), and Fe_3O_4 (Fe) nanoparticles.⁸²

The resulting nano-construct was tested *in vitro* to characterise its capacity to image cells, as well as the resulting cellular viability after prolonged incubation. These tests were able to demonstrate successful uptake of the probe in MCF-7 breast cancer lines after dendrimer mediated dispersion in aqueous solution. The attachment of the cyanine dye facilitated

successful NIR imaging of cells with predominant localisation within the cytoplasm. Despite the multicomponent nature of the nano-probe little to negligible cytotoxicity was observed.

The value of graphene oxide and its reduced derivatives extends beyond its capacity to be readily functionalised, in that it possesses intrinsic near infra-red absorbance properties. As alluded to in the introduction, this is of particular value with respect to imaging and therapeutic applications, primarily because the majority of living tissue is transparent to near infra-red light. Photothermal therapy relies on the capacity of the resulting heat generated from near infra-red light absorption as a means of achieving photoablation which ultimately leads to cancer cell death.⁸³ Therefore, if selective uptake of graphene by cancer cells and tumours can be achieved there is potential for highly efficient imaging and photothermal therapeutic strategies.

Robinson *et al.* employed nano-dimensional reduced graphene oxide with NIR absorbance characteristics that had been further functionalised with RGD (arginylglycylaspartic acid) targeting peptides and an additional cyanine dye (cy5) to further enhance NIR absorption. RGD peptides are known to target $\alpha_v\beta_3$ integrins^e distributed along the membrane of cancer cells.⁸⁴ The derivatisation of the nRGO with RGD via supramolecular PEGylation resulted in a higher confocal fluorescence signal compared to the non-targeted nano-form used as a control (Figure

^e A type of integrin that is a receptor for vitronectin

14).

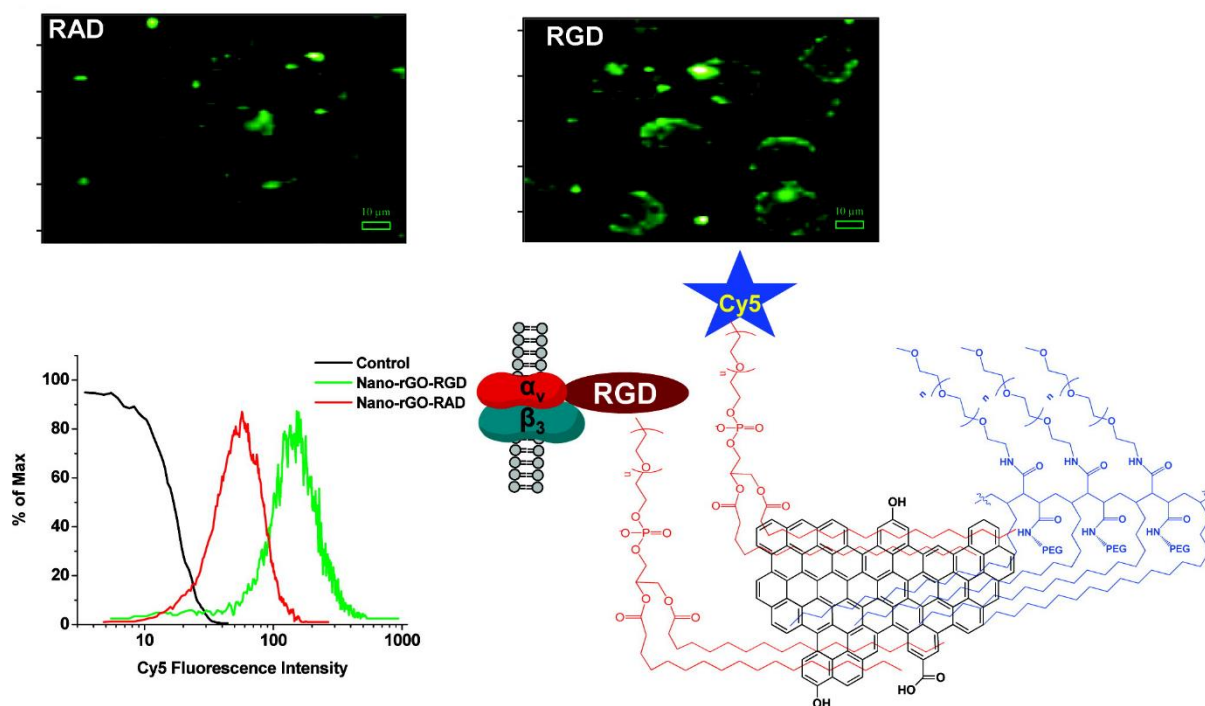


Figure 14: Schematic illustration of peptide and dye immobilisation on GO via PEG derivatisation. (Reprinted with permission from J. T. Robinson, S. M. Tabakman, Y. Liang, H. Wang, H. Sanchez Casalongue, D. Vinh and H. Dai, *Journal of the American Chemical Society*, 2011, 133, 6825-6831. Copyright (2011) American Chemical Society)⁸⁴

This suggested that modifying *nano* surfaces with RGD like peptides is a viable means of enhancing uptake *in vitro*. The nano-conjugates also demonstrated minimal toxicity in human breast cancer cell lines. Furthermore, the targeted nano-construct could also be used to load non-covalently the anti-cancer drug doxorubicin via aromatic stacking reinforced with additional supramolecular interactions. The combination of the targeted nano-conjugate and therapeutic doxorubicin effects allowed for the further reduction in the doses required for the respective photothermal and chemotherapeutic agents.

The need to ensure the retention of the stability of graphene based imaging probes is one of the key drivers behind the regular inclusion of certain dispersing agents such as PEG. However, naphthalene diimides are a particular class of fluorescent aromatic molecules that have demonstrated extensive utility in solubilising carbon allotropes and other aromatic systems.⁸⁵ Naphthalene derived molecules possessing donor- π -acceptor structures have shown promise as platforms for the design of two photon probes for a variety of biological targets. In a study by

Kim *et al.* a series of naphthalene based fluorophores was developed that could visualise and ultimately detect intracellular free metal ions, acidic vesicles and lipid rafts to 300 μm depths inside live tissue samples.⁸⁶ In this work, two photon microscopy was used to demonstrate that the wide two photon cross sections of the naphthalene fluorophores were suitable for obtaining bright two photon images at low concentrations upon binding with intracellular ions/molecules that triggered detectable emission events. The study also demonstrated that such fluorescent probes possessed high cell permeability, selectivity towards cytosolic and membrane bound cellular constituents and an overall high photostability.

1,4,5,8 – Naphthalenediimides (NDIs) are a group of planar, electrochemically active aromatic molecules distinguished by their naphthalene core and diimide nitrogens. Functionalisation via the diimide nitrogens results in naphthalene derivatives possessing variable absorption and emission characteristics. Generally, the attractive optical properties can be attributed to the presence of extended aromatic π conjugated systems. NDIs free of substitution on the naphthalene core tend to absorb only in the UV region.⁸⁷ Extending the π conjugation via the addition of extra aromatic groups on the diimide nitrogens can improve absorption characteristics as is the case with perinones, which have phenylene diamine substituents on the diimide nitrogens and have been used as dyes and pigments as early as the 1950s.⁸⁸ The structure of NDIs also provides them with the ability to form supramolecular assemblies based on donor acceptor interactions. The aromatic and electron deficient characteristics render them with the ability to form face centred aromatic interactions. This particular property opens up avenues for functionalising GO via π - π stacking interactions.⁵⁵ Previous work within the group developed a facile method for the supramolecular complexation of phenylalanine substituted NDI molecules to single walled nanotubes (SWNTs) towards applications in cell imaging and drug delivery.^{85a} This work demonstrated that the NDI molecules could wrap around SWNTs via supramolecular host-guest interactions based on π - π stacking. Two photon fluorescence lifetime imaging (2P-FLIM) demonstrated that the NDI molecule could retain a certain degree of fluorescence upon complexation to the SWNTs (Figure 15).

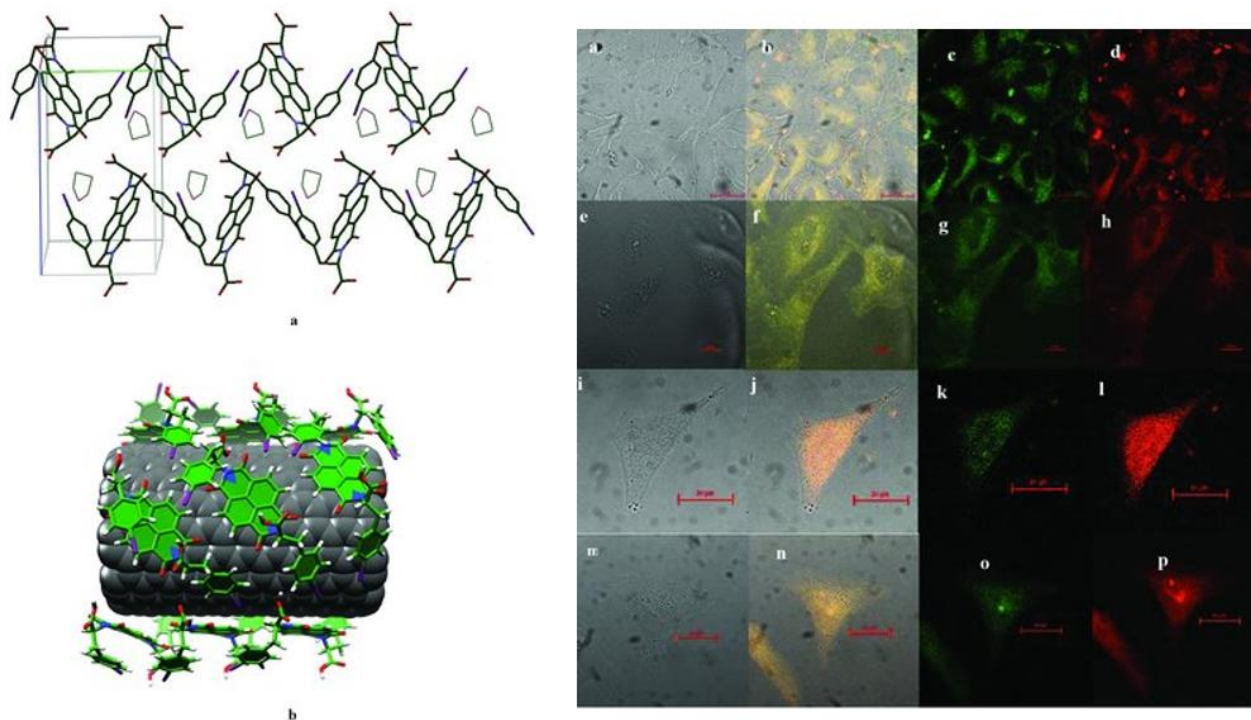
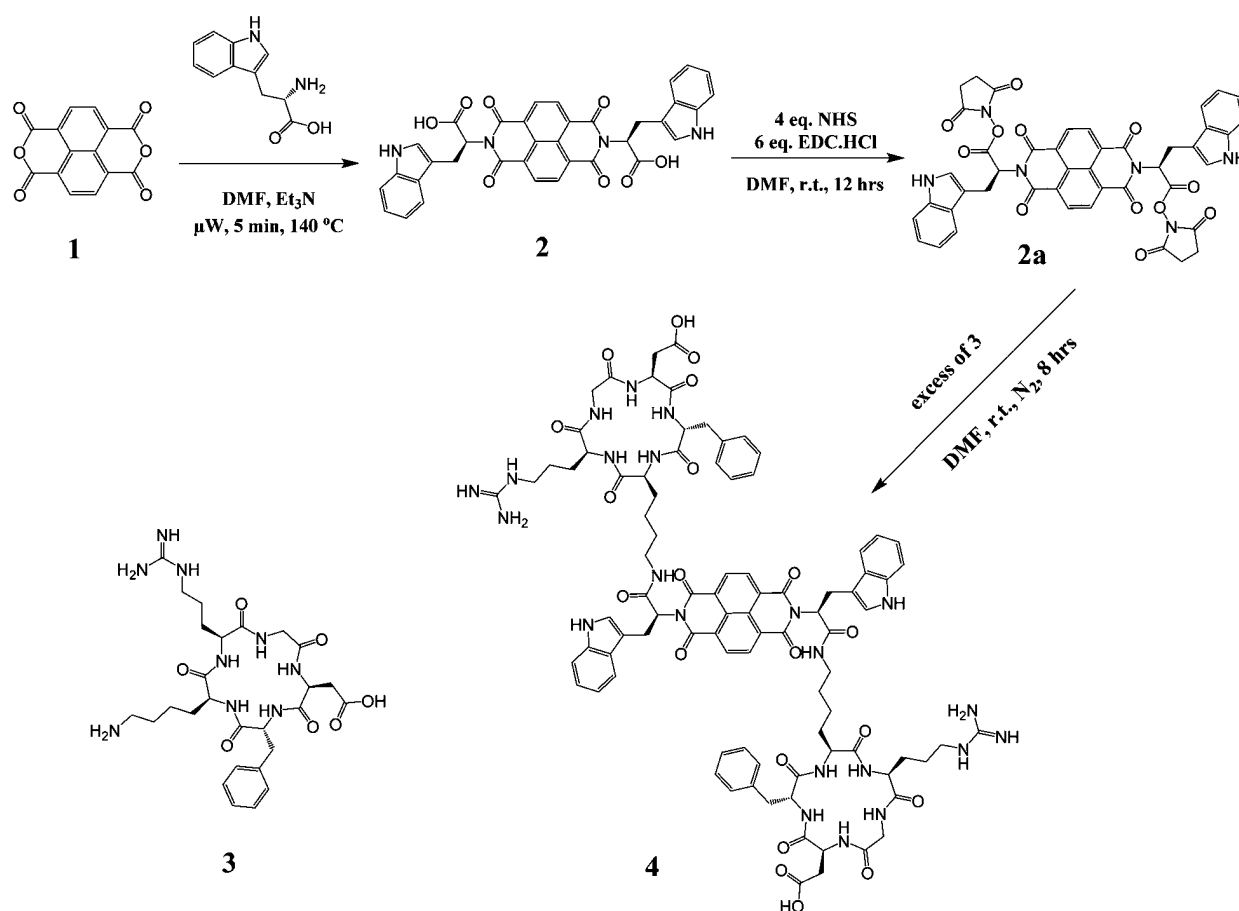


Figure 15: X-ray structure of NDI unit cell fragment proposed structure of a fragment of NDI@SWNT composite: molecular mechanics minimized representation of NDI stacks self-assembled on the surface of a [10:10] SWNT fragment. (Reprinted from Z. Hu, G. D. Pantoş, N. Kuganathan, R. L. Arrowsmith, R. M. J. Jacobs, G. Kociok-Köhn, J. O'Byrne, K. Jurkschat, P. Burgos, R. M. Tyrrell, S. W. Botchway, J. K. M. Sanders and S. I. Pascu, *Advanced Functional Materials*, 2012, 22, 503-518. Copyright (2012), with permission from Wiley)^{85a}

Furthermore, it was demonstrated that functionalisation of SWNTs with the NDIs reduced the cytotoxic properties of the nanotubes and could be internalised within cancerous and non-cancerous cell lines, thus establishing the supramolecular complexes as potential cell imaging probes.

The use of NDI molecules has also been extended to incorporate targeting peptides for more specific imaging strategies for cancer (Scheme 3). We recently developed an NDI based imaging probe for the targeted imaging of cancer cells based on RGD peptide sequence.⁸⁹ Similar to the previous studies mentioned, the RGD sequence was used to target $\alpha_v\beta_3$ integrins more effectively. The over expression of $\alpha_v\beta_3$ integrins has been implicated in the tumour progression and metastasis in a number of cancers during tumour angiogenesis. Consequently, we synthesised a cyclic RGD variant and coupled it to a tryptophan tagged naphthalene diimide. Probing of the fluorescent characteristics of these molecules in PC-3 prostate cancer and non-cancerous FEK-4 cell lines was carried out via confocal and fluorescence lifetime imaging strategies (Figure 16).



Scheme 3: Synthesis of tryptophan-NDI-RGDfK.⁸⁹

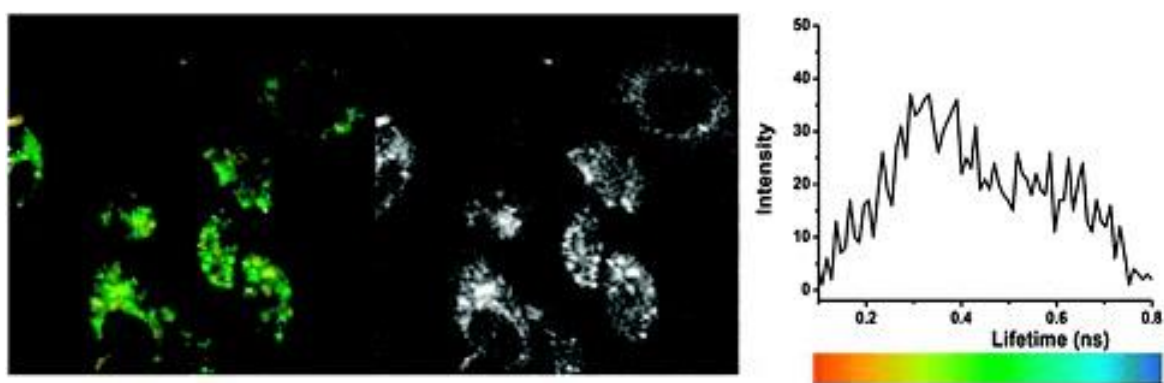


Figure 16: Two-photon laser confocal fluorescence with $\lambda_{\text{ex}} = 810$ nm. A typical micrograph of PC-3 cells incubated for 20 min at 37 °C with tryptophan tagged NDI (100 μM in 1 : 99% DMSO : EMEM) showing compound localisation in vesicular regions in the cytoplasm: lifetime mapping (a), the intensity image and the corresponding average τ lifetime distribution curve and lifetime scale-bar. (Z. Hu, R. L. Arrowsmith, J. A. Tyson, V. Mirabello,

H. Ge, I. M. Eggleston, S. W. Botchway, G. Dan Pantos and S. I. Pascu, *Chemical Communications*, 2015, 51, 6901-6904 - Reproduced by permission of The Royal Society of Chemistry).⁸⁹

These studies revealed the successful uptake of the NDI-peptide conjugate within the cytoplasm of the exposed cells. One of the key findings was a narrowing of the excitation wavelength of the NDI after derivatisation with cyclic RGD to a region of the spectrum that avoided interfering cellular auto fluorescence. This supports the notion that RGD based NDI fluorescent imaging probes can be used to achieve more specific tracing and characterising of $\alpha_v\beta_3$ integrin expression *in vitro*.

The previously mentioned dendrimer immobilisation strategy has also been used to facilitate a synergistic imaging and photothermal therapeutic effect by incorporating targeted phthalocyanine dyes onto GO surfaces. Taratula *et al.* utilised a polypropylene imine dendrimer to immobilise the dye molecules, PEG chains and luteinizing hormone releasing hormone (LHRH) peptide to selectively target ovarian cancer.⁹⁰ This particular design allowed for GO to demonstrate its capacity to facilitate photodynamic therapy, as well as photothermal therapy. Near infra-red radiation of the phthalocyanine allowed for heat generation as energy transferred between the excited dye and the GO. Additionally, the dye could also achieve a triplet excited state which facilitated the formation of reactive oxygen species that created a photodynamic therapeutic effect. These combined strategies allowed for enhanced killing capability (90-95 %) at low dye and graphene concentrations. *In vivo* studies also confirmed the ability of the probe to image cancer tumours in mice.

The exploitation of FRET to enhance photothermal therapy via NIR dyes and GO extends to probe design with much simpler assemblies. Guo *et al.* conjugated the NIR dye cypate^f to GO via a PEG linker. Upon irradiation of the dye, FRET induced an enhanced photothermal effect that was sensitive to pH.⁹¹ The dye-graphene conjugate demonstrated enhanced accumulation capacity in the tumours of mice, despite no specific derivatisation of the probe with a targeting biomolecule (Figure 17).

Additionally, the rapid clearance of the probe from normal tissue has also been demonstrated. *In vivo* studies showed remarkable tumour necrosis and regression 2 days after injection and no tumour re-growth 22 days after injection. This was not the case for control

^f A reactive carbocyanine dye, which is derived from indocyanine green

studies performed in absence of the dye. The GO-PEG construct alone demonstrated reasonable tumour re-growth within a 22 day period post-injection. This confirmed that the effective tumour ablation was ultimately due to the photothermal therapeutic effects facilitated by the presence of the dye and its capacity to undergo FRET with GO.

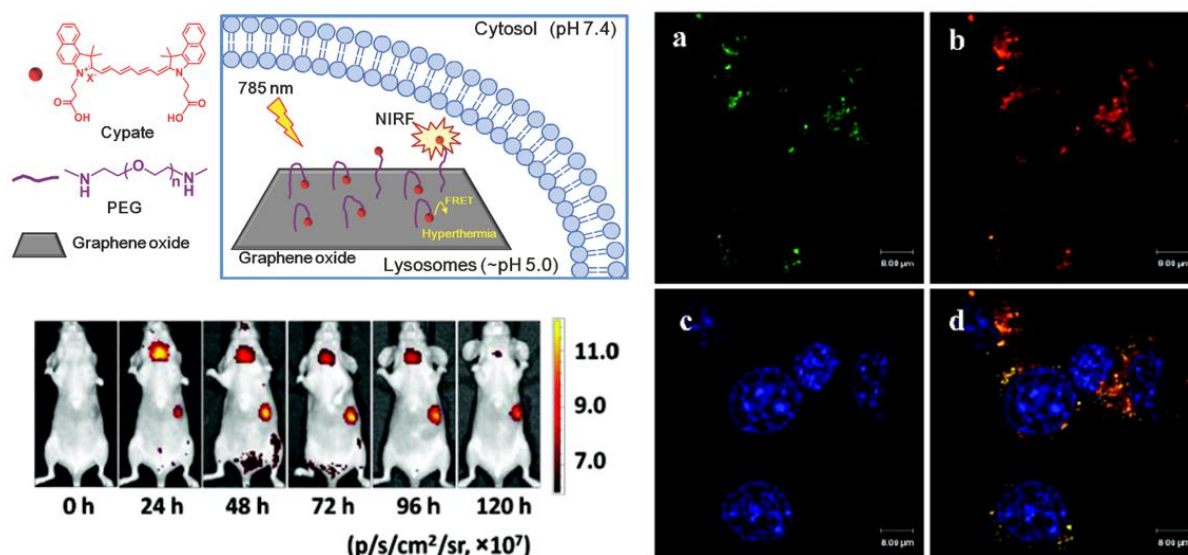


Figure 17: Schematic illustration of GO-Cypate conjugate and *in vitro/vivo* nano-conjugate localisation images. (Reprinted from M. Guo, J. Huang, Y. Deng, H. Shen, Y. Ma, M. Zhang, A. Zhu, Y. Li, H. Hui, Y. Wang, X. Yang, Z. Zhang and H. Chen, *Advanced Functional Materials*, 2015, 25, 59-67. Copyright (2015), with permission from Wiley)⁹¹

The examples described clearly present graphene as a versatile platform for the immobilisation of a broad range of molecules capable of therapeutic and diagnostic goals. These molecules range from a variety of fluorescent dye molecules, solubility enhancing polymers, through to bio-targeting molecules and therapeutic agents. The presence of innate near infra-red absorption characteristics, enhanced by further derivatisation with NIR dyes facilitates photothermal and photodynamic therapeutic effects that have expanded the scope of the benefits of using GO in cancer imaging modalities.

F. Labelling of Graphene Oxide for PET/SPECT Radioactive Imaging and Tracing *in vivo*.

The primary techniques of note when discussing emission tomography based methods of imaging, are Positron Emission Tomography (PET) and Single Photon Emission Computed

Tomography (SPECT). These techniques rely on the use of gamma ray emissions from radioactive materials as a means of visualising a variety of organs, and therefore tumour masses. Unlike some fluorescence based imaging modalities, gamma rays can travel through biological tissue with comparative ease. SPECT agents exploit radiopharmaceuticals that emit a single gamma ray photon per radioactive decay event. However, the radioisotopes used in PET modalities must be positron emitters. During radioactive decay, a positron is emitted from the nucleus of the radioisotope. The positron will then come into contact with an electron leading to annihilation and the subsequent release of two photons (Figure 18). Upon injection of a targeted radiotracer molecule, imaging projections of cross-sectional slices of the organs are generated and a tomographical representation is created from the reconstruction of the constituent images.⁹²

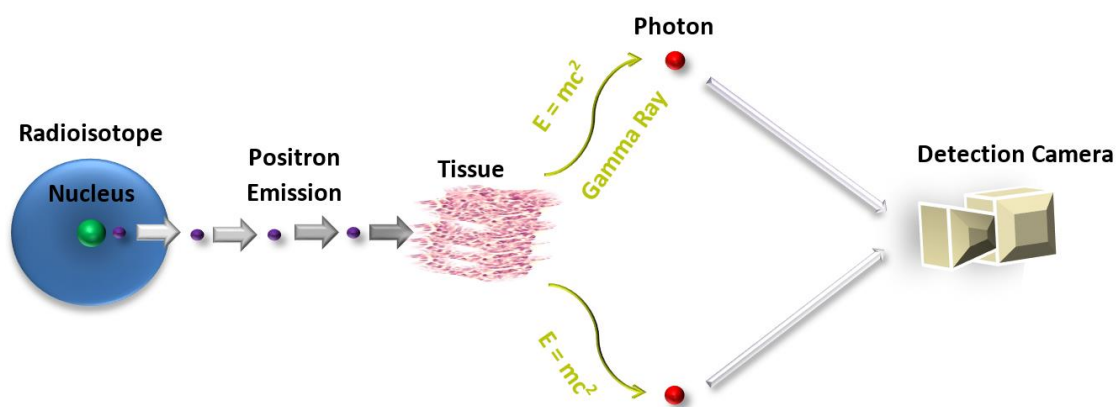


Figure 18: Schematic illustration of processes occurring during PET imaging.

Typical positron emitters and their respective half-lives include ^{18}F ($t_{1/2} = 110$ min), ^{11}C ($t_{1/2} = 20.4$ min), ^{15}O ($t_{1/2} = 124$ s), ^{13}N ($t_{1/2} = 9.96$ min), whereas common single photon emitters are $^{99\text{m}}\text{Tc}$ ($t_{1/2} = 6.02$ h), ^{201}Tl ($t_{1/2} = 73.1$ h), ^{123}I ($t_{1/2} = 13.2$ h), ^{131}I ($t_{1/2} = 8.02$ days), ^{111}In ($t_{1/2} = 2.83$ days), and ^{67}Ga ($t_{1/2} = 78.3$ h).¹⁰ The following section will describe some examples of how these radioisotopes can be used in conjunction with other molecules to facilitate radiolabelled tracing and imaging of cancer *in vivo*.

At this point it is clear that graphene possesses immense potential with respect to the immobilisation of a variety of molecules for a number of imaging and therapeutic applications. Over recent years this has been extended to PET based imaging modalities with ^{66}Ga . ^{66}Ga is of particular interest for GO mediated PET imaging strategies due to its longer half-life (9.5 h) with respect to ^{68}Ga (68 min), a half-life that is relatively complimentary to the *in vivo* kinetics

of GO. One of the more common chelators of gallium radioisotopes is 1,4,7-triaxacyclononane-1,4,7-triacetic acid (NOTA).⁹³ To this end, Hong *et al.*⁹⁴ covalently linked and amine terminated PEG for the immobilisation of ^{66}Ga labelled NOTA that had been previously derivatised with an antibody that targets CD105 (endoglin) expression on the endothelial cells of tumours subject to proliferation.⁹⁵ The resulting pharmacokinetics and capacity for tumour targeting was evaluated via PET imaging in mice carrying murine breast tumours (Figure 19).

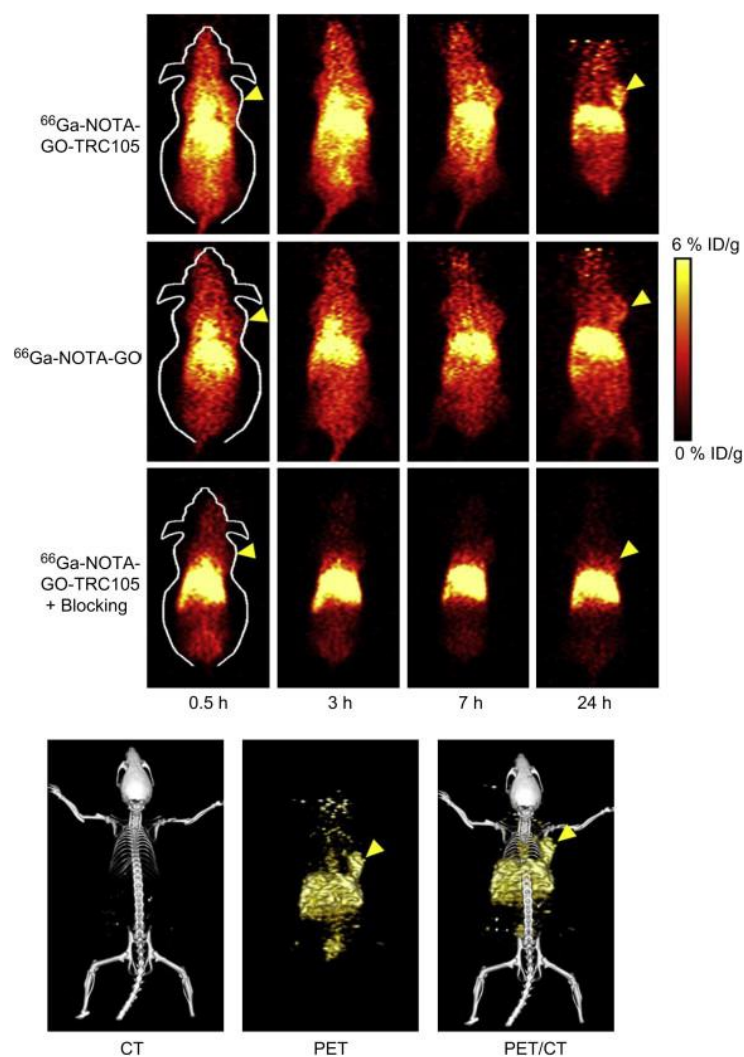


Figure 19: In vivo PET/CT imaging of ^{66}Ga -labeled GO conjugates in 4T1 tumour-bearing mice and representative PET/CT images of ^{66}Ga -NOTA-GO-TRC105 in 4T1 tumour-bearing mice at 3 h post-injection. (Reprinted from H. Hong, Y. Zhang, J. W. Engle, T. R. Nayak, C. P. Theuer, R. J. Nickles, T. E. Barnhart and W. Cai, *Biomaterials*, 2012, 33, 4147-4156. Copyright (2012), with permission from Elsevier)^{94a}

The radiolabelled GO composite showed a rapid and stable tumour accumulation rate over the course of a 24 hour period post injection. This particular study was also able to demonstrate

the suitability of the targeting agent, as histological studies showed there was no GO uptake in the tumours of mice that had been administered with the conjugate without CD105 targeting antibody. The ability to label GO with ^{66}Ga poses considerable opportunity for further study with respect to elucidating the long term outcome and biodistribution of GO due to its superior half-life allowing for more prolonged studies. This may provide more valuable information about the state of tumour development and hence facilitate improved imaging and therapeutic intervention.

This particular strategy towards radiolabelling has also been used to facilitate the inclusion of ^{64}Cu within the targeted GO-NOTA construct.⁹⁶ This immuno-construct possessing a slightly longer half-life (12.7 h) to its ^{66}Ga counterpart showed significant uptake within the tumours, as well as the liver spleen and intestines which is not uncommon for intravenously injected nanomaterials that are cleared through the hepatobiliary system.

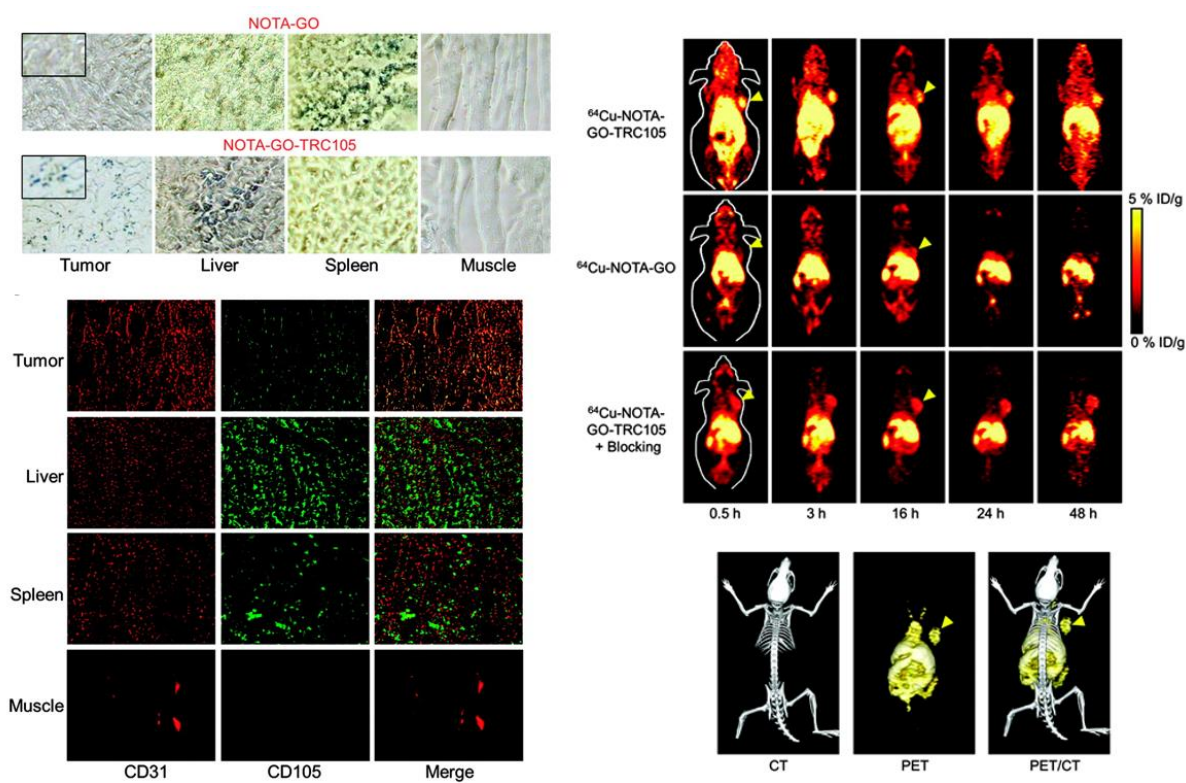


Figure 20: *In vivo* PET/CT imaging of ^{64}Cu -labeled GO conjugates in 4T1 tumour-bearing mice and histological studies on cross-sections from specific organs. (Reprinted with permission from H. Hong, K. Yang, Y. Zhang, J. W. Engle, L. Feng, Y. Yang, T. R. Nayak, S. Goel, J. Bean, C. P. Theuer, T. E. Barnhart, Z. Liu and W. Cai, ACS Nano, 2012, 6, 2361-2370. Copyright (2012) American Chemical Society).^{94b}

Ultimately, these works demonstrate that active and broad labelling of GO can be achieved with a number of radioisotopes provided there is a suitable chelator for the attachment to GO.

The capacity to radiolabel graphene has also extended to the use of ^{125}I isotopes in order to study the localisation and general biodistribution of graphene materials after oral administration or injection. Yang *et al.* used ^{125}I to label PEGylated nanographene oxide (nRGO-PEG).⁹⁷ The *in vivo* biodistribution of labelled GO variants was assessed after oral administration to mice. Considerable levels of radioactivity were observed in the stomach and intestines. However, no radioactivity was monitored in the organs of the mice one week post consumption (Figure 21).

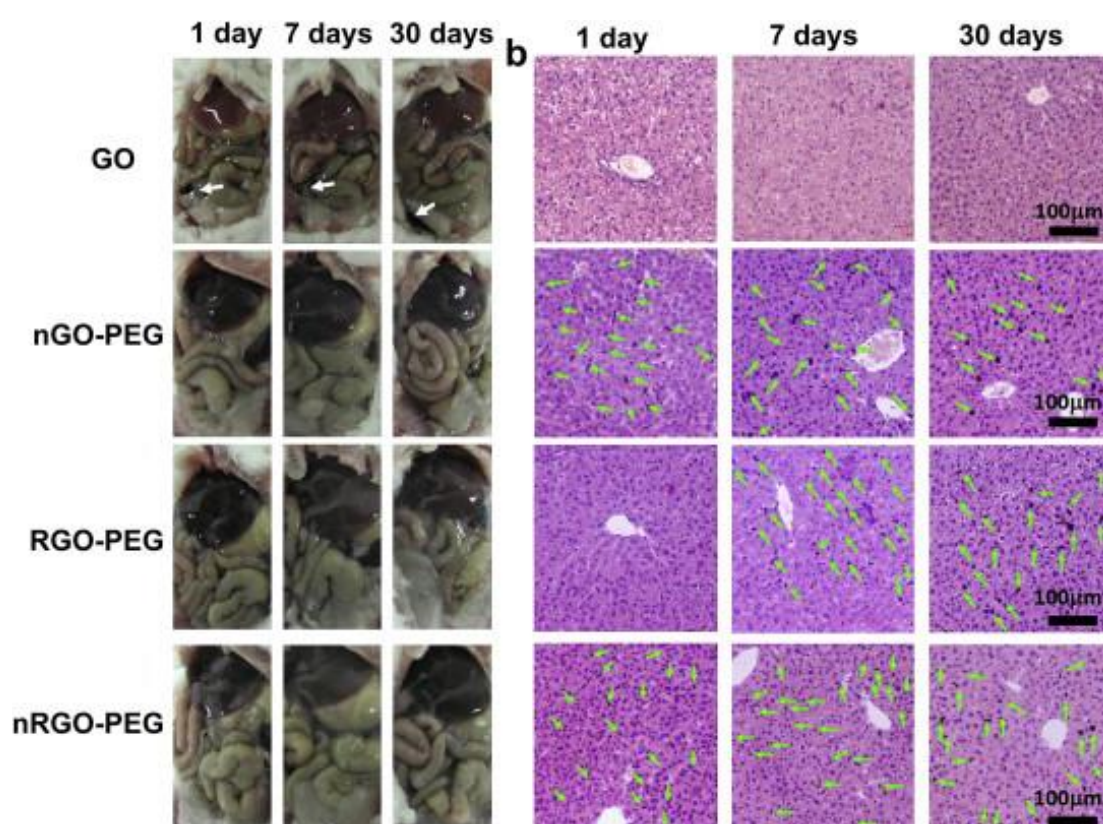


Figure 21: Photos of dissected mice and stained liver slices of mice injected with GO, nGO-PEG, RGO-PEG, and nRGO-PEG at the dose of 50 mg/kg. (Reprinted from K. Yang, H. Gong, X. Shi, J. Wan, Y. Zhang and Z. Liu, *Biomaterials*, 2013, 34, 2787-2795. Copyright (2013), with permission from Elsevier).⁹⁷

The biodistribution of intravenously administered ^{125}I -labelled GO varied in that the nano-construct predominantly localised within the liver and spleen. This study established that the *in vivo* behaviour of graphene nanomaterials was highly dependent on size, surface modification and method of administration. Previous studies by the same group have used ^{125}I

labelling of defect sites or edges of GO in conjunction with polyethylenimine, doxorubicin and plasmid DNA. This allowed for the active tracing of the efficiency of *in vitro* plasmid DNA transfection and combined therapeutic drug delivery.

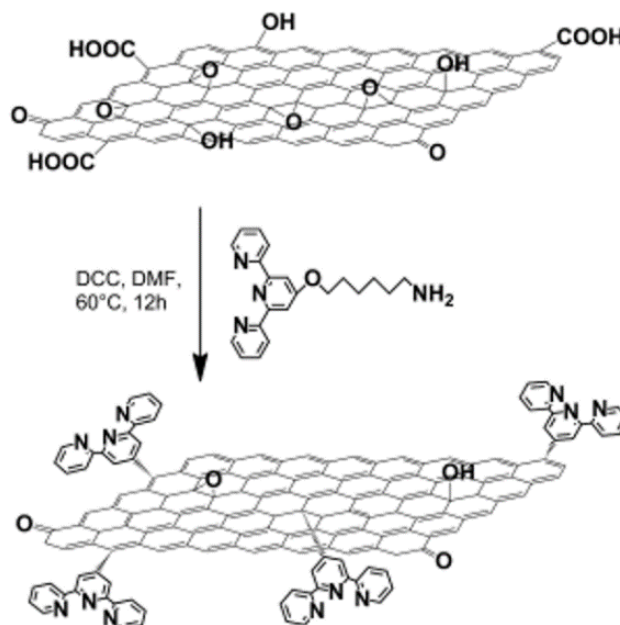
¹¹¹In isotopes are frequently used for the radiolabelling of SPECT imaging applications and are currently being investigated as potential agents for the radio-tracing of GO based materials *in vivo*. Cornelissen *et al.* radiolabelled the metal ion chelator, 2-(4-amino benzyl)-diethylene-triamine pentaacetic acid (p-NH₂-BnDTPA) that was subsequently π - π stacked to the surface of nGO.⁹⁸ The radiolabelled nano-construct was then derivatised with the antibody trastuzumab, an agent for the specific targeting of HER2 receptors over-expressed on breast cancers.

SPECT imaging of the radiolabelled immuno-constructs demonstrated tumour uptake in mouse xenografts. However, a quicker rate of uptake was observed for the radiolabelled probe without GO.

Tumour uptake of the non-specific control was markedly lower than the uptake of its antibody conjugated counterpart. The diminished uptakes of the nano-probe without GO ultimately indicate that there is a complimentary relationship between the nano-graphene and the antibody. This work also demonstrated that another advantage of nGO based radiolabelled immune-constructs in PET/SPECT is associated with the relative ease at which other molecules could be attached, such as anti-cancer drugs and photosensitizers for combined therapy and imaging via multiple modalities.

A significant advance in the field of the GO functionalization has been recently made by Feng, Dani and collaborators.⁹⁹ In 2014, the authors described the use of metallic complexation of 2,2':6', 2''-terpyridine (tpy) with metal ions (Fe and Ru) as a driving force for 3D self-assembling of GO structures. Tpy functionalised GO was obtained by reacting carboxy and epoxy groups of GO with an amine-functionalised terpyridine. The functionalization of GO with N₃ donor ligands may open up new aspects of the technetium and rhenium chemistry. Re and Tc complexes bearing N₃ ligands have been reported in the past. It is known that the three molecules of water in [⁹⁹Tc(CO)₃(H₂O)₃]⁺ can be replaced by a very wide range of terdentate ligands.^{100,101} A GO functionalised with N₃ donor ligands could effectively combine the potential of GO as delivery platform and the use of ⁹⁹Tc as a radiopharmaceutical agent.

Imaging with ^{99m}Tc is still used in the majority of nuclear medical investigations and it has been estimated that around 30-40 millions of technetium scans are annually performed across the world.¹⁰⁰



Scheme 4: Synthetic route towards the functionalisation of GO with terpyridine molecules.

(Reprinted from S. Song, Y. Xue, L. Feng, H. Elbatal, P. Wang, C. N. Moorefield, G. R. Newkome and L. Dai, *Angewandte Chemie International Edition*, 2014, 53, 1415-1419.

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Conclusions and Future Prospects

In the past three decades significant progresses have been made in the field of diagnosis and treatment of cancer tumours. By virtue of advanced bioimaging techniques such as MRI, PET and SPECT, it has become possible to increase the efficacy of cancer screening and detect cancers in tissue and organs even before symptoms appear. MRI techniques have emerged as one of the most promising bioimaging techniques providing detailed images of tissue and organs. MRI agents can modify the relaxation protons in the tissue and organs which induce changes in the MR signal intensity and therefore imaging contrast. Other methods of characterising tumours involve optical fluorescence imaging, which usually involve the use of small organic molecules or nanoparticles that behave as fluorophores. Nuclear medicine tomography is probably the most sensitive method for quantitative measurement *in vivo* and

has been extensively investigated worldwide by chemists and physicians in recent years. One of the benefits of PET and SPECT is that, unlike MRI or fluorescence, these techniques do not necessitate excitation or an external source of incident energy. The source of signal is generated from the radioisotopes employed in the screening analysis. In the past decade, many research groups have focused their attention on developing medical and diagnostic multimodality systems. As nanotechnology has progressively gained serious attention and new surface functionalization methodologies have been established, the challenge of the research community is to incorporate therapeutic and multimodal bioimaging probes in one single *nanosystem*. In this sense, GO and its derivatives, perhaps, represent one of the most promising materials to behave as a platform for bioimaging and vectors for delivery. In this review, we have showed that graphene oxide can be functionalised by using synthetic approaches in order to covalently immobilise chemical species of relevance for imaging and tumour screening. The so called defects of the GO surface can be easily functionalised with fluorophores, biomolecules or organic ligands for metal chelation. The latter aspect could potentially open a new prospective in the use of GO as a scaffold for inorganic radioactive metal complexes having applicability in nuclear medicine tomography.

The functionalization of GO can also be achieved by decorating its surface with biocompatible polymers that, although not directly involved in the imaging process, can be of great benefit to the biocompatibility, distribution and excretion of the probe. The intrinsic aromatic nature of GO can also be used to simplify the chemistry of surface functionalization. Indeed, a great variety of organic molecules, including fluorophores, can self-assemble around GO sheets by virtue of π - π stacking or van der Waals forces to generate supramolecular adducts. The intrinsic near infra-red absorbance properties of GO make this derivative of carbon one of the materials of choice for optical microscopy and imaging. Moreover, the tissue distribution and excretion of chemically functionalised GO have been recently explored and discussed.¹⁰² The work of Bianco and Kostarelos have demonstrated that particular derivatives of GO undergo rapid and significant urine excretion. This work will surely have significant implications for the use of GO *in vivo* and for general medical treatments. Much remains to be developed in the use of GO and carbon allotropes in the context of screening and tumour therapy but it is undisputable that much progress will be achieved considering the growing interest that GO is generating within the scientific community.

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References

1. (a) Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D., Global cancer statistics. *CA: A Cancer Journal for Clinicians* **2011**, *61* (2), 69-90; (b) Statistics, O. f. N., The European age-standardised (AS) incidence rate. *Cancer Statistics Registrations, England (Series MB1)* **2014**, *43*; (c) Wang, Y.; Li, Z.; Wang, J.; Li, J.; Lin, Y., Graphene and graphene oxide: biofunctionalization and applications in biotechnology. *Trends in Biotechnology* **2011**, *29* (5), 205-212.
2. Evan, G. I.; Vousden, K. H., Proliferation, cell cycle and apoptosis in cancer. *Nature* **2001**, *411* (6835), 342-348.
3. Fisher, R.; Pusztai, L.; Swanton, C., Cancer heterogeneity: implications for targeted therapeutics. *Br J Cancer* **2013**, *108* (3), 479-485.
4. (a) Svenson, S., Theranostics: Are We There Yet? *Molecular Pharmaceutics* **2013**, *10* (3), 848-856; (b) Fernandez-Fernandez, A.; Manchanda, R.; McGoron, A., Theranostic Applications of Nanomaterials in Cancer: Drug Delivery, Image-Guided Therapy, and Multifunctional Platforms. *Appl Biochem Biotechnol* **2011**, *165* (7-8), 1628-1651.
5. Sipkins, D. A.; Cheres, D. A.; Kazemi, M. R.; Nevin, L. M.; Bednarski, M. D.; Li, K. C. P., Detection of tumor angiogenesis in vivo by [alpha]v[beta]3-targeted magnetic resonance imaging. *Nat Med* **1998**, *4* (5), 623-626.
6. Kuo, P. H.; Kanal, E.; Abu-Alfa, A. K.; Cowper, S. E., Gadolinium-based MR Contrast Agents and Nephrogenic Systemic Fibrosis. *Radiology* **2007**, *242* (3), 647-649.
7. Thorp-Greenwood, F. L.; Coogan, M. P., Multimodal radio- (PET/SPECT) and fluorescence imaging agents based on metallo-radioisotopes: current applications and prospects for development of new agents. *Dalton Transactions* **2011**, *40* (23), 6129-6143.
8. Yi, X.; Wang, F.; Qin, W.; Yang, X.; Yuan, J., Near-infrared fluorescent probes in cancer imaging and therapy: an emerging field. *International Journal of Nanomedicine* **2014**, *9*, 1347-1365.
9. Yuan, A.; Wu, J.; Tang, X.; Zhao, L.; Xu, F.; Hu, Y., Application of near-infrared dyes for tumor imaging, photothermal, and photodynamic therapies. *Journal of Pharmaceutical Sciences* **2013**, *102* (1), 6-28.
10. Pimlott, S. L.; Sutherland, A., Molecular tracers for the PET and SPECT imaging of disease. *Chemical Society Reviews* **2011**, *40* (1), 149-162.
11. Khalil, M. M.; Tremoleda, J. L.; Bayomy, T. B.; Gsell, W., Molecular SPECT Imaging: An Overview. *International Journal of Molecular Imaging* **2011**, *2011*.
12. Geim, A. K.; Novoselov, K. S., The rise of graphene. *Nat Mater* **2007**, *6* (3), 183-191.
13. Avouris, P., Graphene: Electronic and Photonic Properties and Devices. *Nano Letters* **2010**, *10* (11), 4285-4294.
14. Chen, K.; Song, S.; Xue, D., Beyond graphene: materials chemistry toward high performance inorganic functional materials. *Journal of Materials Chemistry A* **2015**, *3* (6), 2441-2453.

15. Pumera, M., Graphene in biosensing. *Materials Today* **2011**, *14* (7–8), 308-315.
16. Fan, X.; Zhang, G.; Zhang, F., Multiple roles of graphene in heterogeneous catalysis. *Chemical Society Reviews* **2015**, *44* (10), 3023-3035.
17. Bolotin, K. I.; Sikes, K. J.; Jiang, Z.; Klima, M.; Fudenberg, G.; Hone, J.; Kim, P.; Stormer, H. L., Ultrahigh electron mobility in suspended graphene. *Solid State Communications* **2008**, *146* (9–10), 351-355.
18. Lee, C.; Wei, X.; Kysar, J. W.; Hone, J., Measurement of the Elastic Properties and Intrinsic Strength of Monolayer Graphene. *Science* **2008**, *321* (5887), 385-388.
19. Stankovich, S.; Dikin, D. A.; Dommett, G. H. B.; Kohlhaas, K. M.; Zimney, E. J.; Stach, E. A.; Piner, R. D.; Nguyen, S. T.; Ruoff, R. S., Graphene-based composite materials. *Nature* **2006**, *442* (7100), 282-286.
20. Balandin, A. A.; Ghosh, S.; Bao, W.; Calizo, I.; Teweldebrhan, D.; Miao, F.; Lau, C. N., Superior Thermal Conductivity of Single-Layer Graphene. *Nano Letters* **2008**, *8* (3), 902-907.
21. Loh, K. P.; Bao, Q.; Eda, G.; Chhowalla, M., Graphene oxide as a chemically tunable platform for optical applications. *Nat Chem* **2010**, *2* (12), 1015-1024.
22. Shen, H.; Zhang, L.; Liu, M.; Zhang, Z., Biomedical Applications of Graphene. *Theranostics* **2012**, *2* (3), 283-294.
23. Lammers, T.; Aime, S.; Hennink, W. E.; Storm, G.; Kiessling, F., Theranostic Nanomedicine. *Accounts of Chemical Research* **2011**, *44* (10), 1029-1038.
24. (a) van Oosterom, M.; Kreuger, R.; Buckle, T.; Mahn, W.; Bunschoten, A.; Josephson, L.; van Leeuwen, F.; Beekman, F., U-SPECT-BioFluo: an integrated radionuclide, bioluminescence, and fluorescence imaging platform. *EJNMMI Research* **2014**, *4* (1), 56; (b) Berezin, M. Y.; Guo, K.; Teng, B.; Edwards, W. B.; Anderson, C. J.; Vasalatiy, O.; Gandjbakhche, A.; Griffiths, G. L.; Achilefu, S., Radioactivity-Synchronized Fluorescence Enhancement Using a Radionuclide Fluorescence-Quenched Dye. *Journal of the American Chemical Society* **2009**, *131* (26), 9198-9200.
25. Reubi, J. C.; Wenger, S.; Schmuckli-Maurer, J.; Schaer, J.-C.; Gugger, M., Bombesin Receptor Subtypes in Human Cancers: Detection with the Universal Radioligand ¹²⁵I-[d-TYR6, β-ALA11, PHE13, NLE14] Bombesin(6–14). *Clinical Cancer Research* **2002**, *8* (4), 1139-1146.
26. Widengren, J.; Mets, U.; Rigler, R., Fluorescence correlation spectroscopy of triplet states in solution: a theoretical and experimental study. *The Journal of Physical Chemistry* **1995**, *99* (36), 13368-13379.
27. Zhang, W.; Guo, Z.; Huang, D.; Liu, Z.; Guo, X.; Zhong, H., Synergistic effect of chemo-photothermal therapy using PEGylated graphene oxide. *Biomaterials* **2011**, *32* (33), 8555-8561.
28. Castro Neto, A. H.; Guinea, F.; Peres, N. M. R.; Novoselov, K. S.; Geim, A. K., The electronic properties of graphene. *Reviews of Modern Physics* **2009**, *81* (1), 109-162.
29. Zhang, Y.; Tan, Y.-W.; Stormer, H. L.; Kim, P., Experimental observation of the quantum Hall effect and Berry's phase in graphene. *Nature* **2005**, *438* (7065), 201-204.
30. Bonaccorso, F.; Sun, Z.; Hasan, T.; Ferrari, A. C., Graphene photonics and optoelectronics. *Nat Photon* **2010**, *4* (9), 611-622.
31. Abergel, D. S. L.; Apalkov, V.; Berashevich, J.; Ziegler, K.; Chakraborty, T., Properties of graphene: a theoretical perspective. *Advances in Physics* **2010**, *59* (4), 261-482.
32. Kim, K. S.; Zhao, Y.; Jang, H.; Lee, S. Y.; Kim, J. M.; Kim, K. S.; Ahn, J.-H.; Kim, P.; Choi, J.-Y.; Hong, B. H., Large-scale pattern growth of graphene films for stretchable transparent electrodes. *Nature* **2009**, *457* (7230), 706-710.
33. Allen, M. J.; Tung, V. C.; Kaner, R. B., Honeycomb Carbon: A Review of Graphene. *Chemical Reviews* **2009**, *110* (1), 132-145.

34. Dreyer, D. R.; Park, S.; Bielawski, C. W.; Ruoff, R. S., The chemistry of graphene oxide. *Chemical Society Reviews* **2010**, 39 (1), 228-240.
35. Wilson, N. R.; Pandey, P. A.; Beanland, R.; Young, R. J.; Kinloch, I. A.; Gong, L.; Liu, Z.; Suenaga, K.; Rourke, J. P.; York, S. J.; Sloan, J., Graphene Oxide: Structural Analysis and Application as a Highly Transparent Support for Electron Microscopy. *ACS Nano* **2009**, 3 (9), 2547-2556.
36. Novoselov, K. S.; Geim, A. K.; Morozov, S. V.; Jiang, D.; Zhang, Y.; Dubonos, S. V.; Grigorieva, I. V.; Firsov, A. A., Electric Field Effect in Atomically Thin Carbon Films. *Science* **2004**, 306 (5696), 666-669.
37. Brodie, B. C., On the Atomic Weight of Graphite. *Philosophical Transactions of the Royal Society of London* **1859**, 149 (ArticleType: research-article / Full publication date: 1859 /), 249-259.
38. Staudenmaier, L., Verfahren zur Darstellung der Graphitsäure. *Berichte der deutschen chemischen Gesellschaft* **1898**, 31 (2), 1481-1487.
39. Hummers, W. S.; Offeman, R. E., Preparation of Graphitic Oxide. *Journal of the American Chemical Society* **1958**, 80 (6), 1339-1339.
40. Ruess, G., Über das Graphitoxhydroxyd (Graphitoxyd). *Monatshefte für Chemie* **1947**, 76 (3-5), 381-417.
41. Scholz, W.; Boehm, H. P., Untersuchungen am Graphitoxid. VI. Betrachtungen zur Struktur des Graphitoxids. *Zeitschrift für anorganische und allgemeine Chemie* **1969**, 369 (3-6), 327-340.
42. Nakajima, T.; Matsuo, Y., Formation process and structure of graphite oxide. *Carbon* **1994**, 32 (3), 469-475.
43. He, H.; Klinowski, J.; Forster, M.; Lerf, A., A new structural model for graphite oxide. *Chemical Physics Letters* **1998**, 287 (1-2), 53-56.
44. Rodríguez, A. M.; Jiménez, P. S. V., Some new aspects of graphite oxidation at 0°C in a liquid medium. A mechanism proposal for oxidation to graphite oxide. *Carbon* **1986**, 24 (2), 163-167.
45. Zhuang, X.-D.; Chen, Y.; Liu, G.; Li, P.-P.; Zhu, C.-X.; Kang, E.-T.; Noeh, K.-G.; Zhang, B.; Zhu, J.-H.; Li, Y.-X., Conjugated-Polymer-Functionalized Graphene Oxide: Synthesis and Nonvolatile Rewritable Memory Effect. *Advanced Materials* **2010**, 22 (15), 1731-1735.
46. Liu, F.; Choi, J. Y.; Seo, T. S., Graphene oxide arrays for detecting specific DNA hybridization by fluorescence resonance energy transfer. *Biosensors and Bioelectronics* **2010**, 25 (10), 2361-2365.
47. Paula, A. A. P. M.; Gil, G.; Sandra, C.; Nuno, A.; Manoj, K. S.; José, G.; Antonio, C. M. S., Functionalized Graphene Nanocomposites. 2011.
48. Hermanson, G. T., *Bioconjugate Techniques*. Elsevier Science: 2010.
49. Liu, Z.; Robinson, J. T.; Sun, X.; Dai, H., PEGylated Nanographene Oxide for Delivery of Water-Insoluble Cancer Drugs. *Journal of the American Chemical Society* **2008**, 130 (33), 10876-10877.
50. Xu, Y.; Liu, Z.; Zhang, X.; Wang, Y.; Tian, J.; Huang, Y.; Ma, Y.; Zhang, X.; Chen, Y., A Graphene Hybrid Material Covalently Functionalized with Porphyrin: Synthesis and Optical Limiting Property. *Advanced Materials* **2009**, 21 (12), 1275-1279.
51. Jiang, Y.; Zhang, Q.; Li, F.; Niu, L., Glucose oxidase and graphene bionanocomposite bridged by ionic liquid unit for glucose biosensing application. *Sensors and Actuators B: Chemical* **2012**, 161 (1), 728-733.
52. Mei, Q.; Zhang, K.; Guan, G.; Liu, B.; Wang, S.; Zhang, Z., Highly efficient photoluminescent graphene oxide with tunable surface properties. *Chemical Communications* **2010**, 46 (39), 7319-7321.

53. Chien, C.-T.; Li, S.-S.; Lai, W.-J.; Yeh, Y.-C.; Chen, H.-A.; Chen, I. S.; Chen, L.-C.; Chen, K.-H.; Nemoto, T.; Isoda, S.; Chen, M.; Fujita, T.; Eda, G.; Yamaguchi, H.; Chhowalla, M.; Chen, C.-W., Tunable Photoluminescence from Graphene Oxide. *Angewandte Chemie International Edition* **2012**, *51* (27), 6662-6666.
54. Hunter, C. A.; Sanders, J. K. M., The nature of π - π interactions. *Journal of the American Chemical Society* **1990**, *112* (14), 5525-5534.
55. Martinez, C. R.; Iverson, B. L., Rethinking the term "pi-stacking". *Chemical Science* **2012**, *3* (7), 2191-2201.
56. Linares, J.; Matesanz, M. C.; Vila, M.; Feito, M. J.; Gonçalves, G.; Vallet-Regí, M.; Marques, P. A. A. P.; Portolés, M. T., Endocytic Mechanisms of Graphene Oxide Nanosheets in Osteoblasts, Hepatocytes and Macrophages. *ACS Applied Materials & Interfaces* **2014**, *6* (16), 13697-13706.
57. Kerr, M. C.; Teasdale, R. D., Defining Macropinocytosis. *Traffic* **2009**, *10* (4), 364-371.
58. Sun, X.; Liu, Z.; Welsher, K.; Robinson, J.; Goodwin, A.; Zaric, S.; Dai, H., Nano-graphene oxide for cellular imaging and drug delivery. *Nano Res.* **2008**, *1* (3), 203-212.
59. Johnson, I. D., *The Molecular Probes Handbook: A Guide to Fluorescent Probes and Labeling Technologies, 11th Edition*. Life Technologies Corporation: 2010.
60. Xu, H.; Chen, R.; Sun, Q.; Lai, W.; Su, Q.; Huang, W.; Liu, X., Recent progress in metal-organic complexes for optoelectronic applications. *Chemical Society Reviews* **2014**, *43* (10), 3259-3302.
61. Lakowicz, J. R., *Principles of Fluorescence Spectroscopy*. Springer: 2007.
62. Ferrari, M., Cancer nanotechnology: opportunities and challenges. *Nat Rev Cancer* **2005**, *5* (3), 161-171.
63. Thundimadathil, J., Cancer Treatment Using Peptides: Current Therapies and Future Prospects. *Journal of Amino Acids* **2012**, *2012*, 13.
64. Aina, O. H.; Sroka, T. C.; Chen, M.-L.; Lam, K. S., Therapeutic cancer targeting peptides. *Peptide Science* **2002**, *66* (3), 184-199.
65. Reubi, J. C., Peptide Receptors as Molecular Targets for Cancer Diagnosis and Therapy. *Endocrine Reviews* **2003**, *24* (4), 389-427.
66. Zhang, Y.; Wu, C.; Guo, S.; Zhang, J., Interactions of graphene and graphene oxide with proteins and peptides. In *Nanotechnology Reviews*, 2013; Vol. 2, p 27.
67. Cui, Y.; Kim, S. N.; Jones, S. E.; Wissler, L. L.; Naik, R. R.; McAlpine, M. C., Chemical Functionalization of Graphene Enabled by Phage Displayed Peptides. *Nano Letters* **2010**, *10* (11), 4559-4565.
68. (a) Kim, S. N.; Kuang, Z.; Slocik, J. M.; Jones, S. E.; Cui, Y.; Farmer, B. L.; McAlpine, M. C.; Naik, R. R., Preferential Binding of Peptides to Graphene Edges and Planes. *Journal of the American Chemical Society* **2011**, *133* (37), 14480-14483; (b) Han, T. H.; Lee, W. J.; Lee, D. H.; Kim, J. E.; Choi, E.-Y.; Kim, S. O., Peptide/Graphene Hybrid Assembly into Core/Shell Nanowires. *Advanced Materials* **2010**, *22* (18), 2060-2064.
69. Katoch, J.; Kim, S. N.; Kuang, Z.; Farmer, B. L.; Naik, R. R.; Tatulian, S. A.; Ishigami, M., Structure of a Peptide Adsorbed on Graphene and Graphite. *Nano Letters* **2012**, *12* (5), 2342-2346.
70. Qin, W.; Li, X.; Bian, W.-W.; Fan, X.-J.; Qi, J.-Y., Density functional theory calculations and molecular dynamics simulations of the adsorption of biomolecules on graphene surfaces. *Biomaterials* **2010**, *31* (5), 1007-1016.
71. (a) Roman, T.; Diño, W. A.; Nakanishi, H.; Kasai, H., Glycine adsorption on single-walled carbon nanotubes. *Thin Solid Films* **2006**, *509* (1-2), 218-222; (b) Guo, Y.-n.; Lu, X.; Weng, J.; Leng, Y., Density Functional Theory Study of the Interaction of Arginine-Glycine-

Aspartic Acid with Graphene, Defective Graphene, and Graphene Oxide. *The Journal of Physical Chemistry C* **2013**, *117* (11), 5708-5717.

72. Gianese, G.; Rosato, V.; Cleri, F.; Celino, M.; Morales, P., Atomic-Scale Modeling of the Interaction between Short Polypeptides and Carbon Surfaces. *The Journal of Physical Chemistry B* **2009**, *113* (35), 12105-12112.

73. Zhang, M.; Yin, B.-C.; Wang, X.-F.; Ye, B.-C., Interaction of peptides with graphene oxide and its application for real-time monitoring of protease activity. *Chemical Communications* **2011**, 47 (8), 2399-2401.

74. Wang, H.; Zhang, Q.; Chu, X.; Chen, T.; Ge, J.; Yu, R., Graphene Oxide–Peptide Conjugate as an Intracellular Protease Sensor for Caspase-3 Activation Imaging in Live Cells. *Angewandte Chemie International Edition* **2011**, *50* (31), 7065-7069.

75. Ji, H.; Guan, Y.; Wu, L.; Ren, J.; Miyoshi, D.; Sugimoto, N.; Qu, X., A fluorescent probe for detection of an intracellular prognostic indicator in early-stage cancer. *Chemical Communications* **2015**, 51 (8), 1479-1482.

76. Vicini, F. A.; Vargas, C.; Abner, A.; Kestin, L.; Horwitz, E.; Martinez, A., LIMITATIONS IN THE USE OF SERUM PROSTATE SPECIFIC ANTIGEN LEVELS TO MONITOR PATIENTS AFTER TREATMENT FOR PROSTATE CANCER. *The Journal of Urology* **2005**, *173* (5), 1456-1462.

77. (a) Mikolajczyk, S. D.; Rittenhouse, H. G., Pro PSA: a more cancer specific form of prostate specific antigen for the early detection of prostate cancer. *The Keio Journal of Medicine* **2003**, *52* (2), 86-91; (b) Sun, C.; Su, K.-H.; Valentine, J.; Rosa-Bauza, Y. T.; Ellman, J. A.; Elboudwarej, O.; Mukherjee, B.; Craik, C. S.; Shuman, M. A.; Chen, F. F.; Zhang, X., Time-Resolved Single-Step Protease Activity Quantification Using Nanoplasmonic Resonator Sensors. *ACS Nano* **2010**, *4* (2), 978-984; (c) Zhao, N.; He, Y.; Mao, X.; Sun, Y.; Zhang, X.; Li, C.-z.; Lin, Y.; Liu, G., Electrochemical assay of active prostate-specific antigen (PSA) using ferrocene-functionalized peptide probes. *Electrochemistry Communications* **2010**, *12* (3), 471-474; (d) Denmeade, S. R.; Lou, W.; Lövgren, J.; Malm, J.; Lilja, H.; Isaacs, J. T., Specific and Efficient Peptide Substrates for Assaying the Proteolytic Activity of Prostate-specific Antigen. *Cancer Research* **1997**, *57* (21), 4924-4930.

78. Feng, T.; Feng, D.; Shi, W.; Li, X.; Ma, H., A graphene oxide-peptide fluorescence sensor for proteolytically active prostate-specific antigen. *Molecular BioSystems* **2012**, *8* (5), 1441-1445.

79. Reubi, J. C.; Laissue, J.; Krenning, E.; Lamberts, S. W. J., Somatostatin receptors in human cancer: Incidence, characteristics, functional correlates and clinical implications. *The Journal of Steroid Biochemistry and Molecular Biology* **1992**, *43* (1–3), 27-35.

80. Bianying, F.; Linjie, G.; Lihua, W.; Fan, L.; Jianxin, L.; Jimin, G.; Chunhai, F.; Qing, H., A Graphene Oxide-Based Fluorescent Biosensor for the Analysis of Peptide–Receptor Interactions and Imaging in Somatostatin Receptor Subtype 2 Overexpressed Tumor Cells. *Analytical Chemistry* **2013**, *85* (16), 7732-7737.

81. Šebestík, J.; Reiniš, M.; Ježek, J., Dendrimers in Nanoscience and Nanotechnology. In *Biomedical Applications of Peptide-, Glyco- and Glycopeptide Dendrimers, and Analogous Dendrimeric Structures*, Springer Vienna: 2012; pp 115-129.

82. Prateek, S. W.; Shashwat, S. B.; Archana, J.-B.; Russel, R. M.; Khushbu, R. Z.; Jayant, K.; Misra, R. D. K., Cellular imaging using biocompatible dendrimer-functionalized graphene oxide-based fluorescent probe anchored with magnetic nanoparticles. *Nanotechnology* **2012**, *23* (41), 415101.

83. Huang, X.; El-Sayed, M. A., Plasmonic photo-thermal therapy (PPTT). *Alexandria Journal of Medicine* **2011**, *47* (1), 1-9.

84. Robinson, J. T.; Tabakman, S. M.; Liang, Y.; Wang, H.; Sanchez Casalongue, H.; Vinh, D.; Dai, H., Ultrasmall Reduced Graphene Oxide with High Near-Infrared Absorbance for Photothermal Therapy. *Journal of the American Chemical Society* **2011**, *133* (17), 6825-6831.
85. (a) Hu, Z.; Pantoş, G. D.; Kuganathan, N.; Arrowsmith, R. L.; Jacobs, R. M. J.; Kociok-Köhn, G.; O'Byrne, J.; Jurkschat, K.; Burgos, P.; Tyrrell, R. M.; Botchway, S. W.; Sanders, J. K. M.; Pascu, S. I., Interactions Between Amino Acid-Tagged Naphthalenediimide and Single Walled Carbon Nanotubes for the Design and Construction of New Bioimaging Probes. *Advanced Functional Materials* **2012**, *22* (3), 503-518; (b) Pantoş, G. D.; Wietor, J.-L.; Sanders, J. K. M., Filling Helical Nanotubes with C60. *Angewandte Chemie International Edition* **2007**, *46* (13), 2238-2240.
86. Kim, H. M.; Cho, B. R., Two-Photon Probes for Intracellular Free Metal Ions, Acidic Vesicles, And Lipid Rafts in Live Tissues. *Accounts of Chemical Research* **2009**, *42* (7), 863-872.
87. Alp, S.; Erten, Ş.; Karapire, C.; Köz, B.; Doroshenko, A. O.; İçli, S., Photoinduced energy–electron transfer studies with naphthalene diimides. *Journal of Photochemistry and Photobiology A: Chemistry* **2000**, *135* (2–3), 103-110.
88. Thalacker, C.; Röger, C.; Würthner, F., Synthesis and Optical and Redox Properties of Core-Substituted Naphthalene Diimide Dyes. *The Journal of Organic Chemistry* **2006**, *71* (21), 8098-8105.
89. Hu, Z.; Arrowsmith, R. L.; Tyson, J. A.; Mirabello, V.; Ge, H.; Eggleston, I. M.; Botchway, S. W.; Dan Pantos, G.; Pascu, S. I., A fluorescent Arg-Gly-Asp (RGD) peptide-naphthalenediimide (NDI) conjugate for imaging integrin $\alpha_v\beta_3$ in vitro. *Chemical Communications* **2015**, *51* (32), 6901-6904.
90. Taratula, O.; Patel, M.; Schumann, C.; Naleway, M. A.; Pang, A. J.; He, H.; Taratula, O., Phthalocyanine-loaded graphene nanoplatfrom for imaging-guided combinatorial phototherapy. *International Journal of Nanomedicine* **2015**, *10*, 2347-2362.
91. Guo, M.; Huang, J.; Deng, Y.; Shen, H.; Ma, Y.; Zhang, M.; Zhu, A.; Li, Y.; Hui, H.; Wang, Y.; Yang, X.; Zhang, Z.; Chen, H., pH-Responsive Cyanine-Grafted Graphene Oxide for Fluorescence Resonance Energy Transfer-Enhanced Photothermal Therapy. *Advanced Functional Materials* **2015**, *25* (1), 59-67.
92. Wernick, M. N.; Aarsvold, J. N., *Emission Tomography: The Fundamentals of PET and SPECT*. Elsevier Science: 2004.
93. Morfin, J.-F.; Tóth, É., Kinetics of Ga(NOTA) Formation from Weak Ga-Citrate Complexes. *Inorganic Chemistry* **2011**, *50* (20), 10371-10378.
94. (a) Hong, H.; Zhang, Y.; Engle, J. W.; Nayak, T. R.; Theuer, C. P.; Nickles, R. J.; Barnhart, T. E.; Cai, W., In vivo targeting and positron emission tomography imaging of tumor vasculature with ^{66}Ga -labeled nano-graphene. *Biomaterials* **2012**, *33* (16), 4147-4156; (b) Hong, H.; Yang, K.; Zhang, Y.; Engle, J. W.; Feng, L.; Yang, Y.; Nayak, T. R.; Goel, S.; Bean, J.; Theuer, C. P.; Barnhart, T. E.; Liu, Z.; Cai, W., In Vivo Targeting and Imaging of Tumor Vasculature with Radiolabeled, Antibody-Conjugated Nanographene. *ACS Nano* **2012**, *6* (3), 2361-2370.
95. DUFF, S. E.; LI, C.; GARLAND, J. M.; KUMAR, S., CD105 is important for angiogenesis: evidence and potential applications. *The FASEB Journal* **2003**, *17* (9), 984-992.
96. Shi, S.; Yang, K.; Hong, H.; Valdovinos, H. F.; Nayak, T. R.; Zhang, Y.; Theuer, C. P.; Barnhart, T. E.; Liu, Z.; Cai, W., Tumor vasculature targeting and imaging in living mice with reduced graphene oxide. *Biomaterials* **2013**, *34* (12), 3002-3009.
97. Yang, K.; Gong, H.; Shi, X.; Wan, J.; Zhang, Y.; Liu, Z., In vivo biodistribution and toxicology of functionalized nano-graphene oxide in mice after oral and intraperitoneal administration. *Biomaterials* **2013**, *34* (11), 2787-2795.

98. Cornelissen, B.; Able, S.; Kersemans, V.; Waghorn, P. A.; Myhra, S.; Jurkshat, K.; Crossley, A.; Vallis, K. A., Nanographene oxide-based radioimmunoconstructs for in vivo targeting and SPECT imaging of HER2-positive tumors. *Biomaterials* **2013**, *34* (4), 1146-1154.
99. Song, S.; Xue, Y.; Feng, L.; Elbatal, H.; Wang, P.; Moorefield, C. N.; Newkome, G. R.; Dai, L., Reversible Self-Assembly of Terpyridine-Functionalized Graphene Oxide for Energy Conversion. *Angewandte Chemie International Edition* **2014**, *53* (5), 1415-1419.
100. Dilworth, J. R.; Pascu, S. I., The Radiopharmaceutical Chemistry of Technetium and Rhenium. In *The Chemistry of Molecular Imaging*, John Wiley & Sons, Inc: 2014; pp 137-164.
101. (a) Maresca, K. P.; Hillier, S. M.; Femia, F. J.; Zimmerman, C. N.; Levadala, M. K.; Banerjee, S. R.; Hicks, J.; Sundararajan, C.; Valliant, J.; Zubieta, J.; Eckelman, W. C.; Joyal, J. L.; Babich, J. W., Comprehensive Radiolabeling, Stability, and Tissue Distribution Studies of Technetium-99m Single Amino Acid Chelates (SAAC). *Bioconjugate Chemistry* **2009**, *20* (8), 1625-1633; (b) Ferreira, C. L.; Marques, F. L. N.; Okamoto, M. R. Y.; Otake, A. H.; Sugai, Y.; Mikata, Y.; Storr, T.; Bowen, M.; Yano, S.; Adam, M. J.; Chammas, R.; Orvig, C., Cationic technetium and rhenium complexes with pendant carbohydrates. *Applied Radiation and Isotopes* **2010**, *68* (6), 1087-1093.
102. Jasim, D. A.; Menard-Moyon, C.; Begin, D.; Bianco, A.; Kostarelos, K., Tissue distribution and urinary excretion of intravenously administered chemically functionalized graphene oxide sheets. *Chemical Science* **2015**, *6* (7), 3952-3964.

List of abbreviations

* 2P-FLIM	Two photon fluorescence lifetime imaging microscopy
* AFM:	Atomic force microscopy
* AOC:	Anti-octreotide
* CD105:	Endoglin
* CDK:	Cyclin dependant kinase
* CT:	Commutated tomography
* CVD:	Chemical vapour deposition
* Cy5:	Cyanine dye
* DCC:	<i>N,N'</i> -dicyclohexylcarbodiimide
* DFT:	Density functional theory
* DNA:	Deoxyribonucleic acid
* DOX:	Doxorubicin
* EDC:	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
* FAM:	Fluorescein amidite
* FITC:	Fluorescein isothiocyanate
* FOC:	Fluorescein isothiocyanate dye labelled octreotide
* FRET:	Forster Resonance Energy Transfer
* GO:	Graphene oxide
* GS:	Graphene-silica
* GSH:	Glutathione
* HATU:	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo [4,5-b]pyridinium 3-oxid hexafluorophosphate
* HER2:	Human epidermal growth factor receptor 2 oncogen
* IL:	Ionic liquid
* LHRH:	Luteinizing hormone releasing hormone
* MRI:	Magnetic resonance imaging
* NDI:	Naphthalenediimide
* nGO:	Nano graphene oxide
* NHS:	N-hydroxysuccinimide
* NIR:	Near infrared
* NMR:	Nuclear Magnetic Resonance
* NOTA:	1,4,7-triaxacyclononane-1,4,7-triacetic acid
* nRGO:	Nano reduced graphene oxide
* PAMAM G4:	A polyamidoamine dendrimer of fourth generation
* PEG:	Polyethylene glycol
* PEG-RWIMYF:	Polyethylene glycol modified specific hexapeptide
* PET:	Positron Emission Tomography
* p-NH ₂ -BnDTPA	2-(4-amino benzyl)-diethylene-triamine pentaacetic acid
* PSA:	Prostate Specific Antigen
* RGD:	Arginylglycylaspartic acid
* RNA:	Ribonucleic acid

* SPECT:	Single photon emission computed tomography
* ssDNA:	Single-stranded deoxyribonucleic acid
* STS:	Staurosporine
* SWNT:	Single walled nanotube
* TEM:	Transmission electron microscopy
* Tpy:	2,2':6', 2''-terpyridine
* TRC105:	Anti-endoglin antibody